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Title:
A Systematic review and Meta-Analysis on altered brain structure in patients born with non-syndromic cleft lip and/or palate.

A Systematic review and Meta-Analysis on altered brain structure in patients born with non-syndromic cleft lip and/or palate.

By Nadine Homoud

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Doctor of Dental Surgery by advanced study in Orthodontics in the Faculty of Health Sciences.

July 2020

Words count (25203)

Abstract

Title: A Systematic review and meta-analysis on altered brain structure in patients born with non-syndromic cleft lip and/or palate.

Objective: To determine if there is evidence for a relationship between the presence of a non-syndromic cleft lip and/or palate (NSCLP) and altered brain structure in cleft affected individuals.

Design: Electronic database (MEDLINE; EMBASE; Cochrane library) and manual searches were performed and were limited to English language texts published between1st January 1969 until February 2020. Data extraction and risk of bias assessment were done independently by two reviewers. A meta-analysis on seven publications was performed using a random effects model.

Main outcome Measure: Comparison of brain structure between patients born with cleft lip and/or palate and unaffected healthy individuals.

Results: The systematic review comprised 9 studies of which seven were included in the meta-analysis. The latter comprised 360 individuals with NSCLP compared to unaffected controls. There was a statistically significant reduction in the overall intracranial volume and the total cerebellar volume in the cleft group compared to controls (Hedges g and 95% confidence intervals -0.38 (CI -0.71, -0.06) and -0.77 (CI -0.94, -0.60) respectively. For the frontal cortex and straight gyrus; total cerebral volume; and cortical grey matter there was no statistically significant difference between the cleft affected and unaffected individuals.

Conclusion: In individuals with non-syndromic cleft lip and/or palate, the overall intracranial volume and the total cerebellar volume are both statistically significantly smaller than in unaffected controls.

Dedication and Acknowledgements

I would like to express my deep appreciation to Professor Anthony Ireland and Professor Jonathan Sandy, my research supervisors, for their patient guidance, valuable support and expert advice throughout this project.

Many thanks to Professor Martyn Sherriff for his expert help in the statistical analysis of this project.

I would also like to extend my thanks to my colleagues for their encouragement.

To my parents and brother, your love and endless inspiration gave me power to overcome any obstacle, for that I am forever grateful and this research is dedicated to you.

To my friends who are my chosen family, thank you for your continuous reassurance and for always being there.

I heartily thank God for his endless blessings and abundant guidance to complete this study.

Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: Nadine Homoud DATE: 07/07/2020

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List of Abbreviations

CLP	Cleft lip and/or palate
CPO	Isolated cleft palate
CL/P	Cleft lip, alveolus with or without cleft palate
NSCLP	Nonsyndromic cleft lip and palate
TGFα	Transforming growth factor alpha
MSX1	Msx homeobox 1
ENT	Ear, Nose, Throat surgeon
IQ	Intelligence quotient
FGF8	Fibroblast growth factor 8
SHH	Sonic hedgehog
MRI	Magnetic resonance imaging
PET	Positron emission tomography
PECO	Population, Exposure, Comparator, Outcome
NH	Nadine Homoud
ZA	Zainab Al-Saffar
AI	Anthony Ireland
CASP	Critical Appraisal Risks Programme
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
BRAINS	Brain research: Analysis of Images, Network and Systems
CSF	Cerebrospinal fluid
VFC	Ventral Frontal Cortex
PSYCH	Psychiatric Symptom You Currently Have assessment
OFC	Orbitofrontal cortex
SG	Straight gyrus
MANOVA	Multivariate analysis of variance
3D	3-dimensional
ANCOVA	Analysis for Covariance
CI	Confidence intervals
NSCL	Nonsyndromic cleft lip only
CLO	Clefts of the lip only
NSCP	Nonsyndromic cleft palate only

1. Introduction

Cleft lip and/or palate (CLP) is a common birth defect affecting the orofacial region, with incidence rates between 1 and 2 in every 1000 live births (Mossey *et al.,* 2009). The incidence is higher (approximately 50%) if all embryos are included and varies with ethnicity, gender, and socioeconomic status (Bender, 2000). Up to 30 percent of children born with cleft lip and/or palate have the condition as part of a syndrome, whereas in the remaining 70 percent it is an isolated anomaly (Calzolari *et al.,* 2007). The aetiology of cleft lip and/or palate and cleft palate only (CPO) is multifactorial and is commonly a result of an interplay between genetic and environmental factors (Murray, 2002).

Children born with CLP may suffer from a variety of problems through childhood and into adult life. These can include initial difficulties with feeding, speech and hearing, appearance, social exclusion, and bullying. These issues can compound and may have an impact on their educational attainment. As the child continues to develop, the appearance of the cleft may also influence their psychological well-being (Mossey *et al.,* 2009).

The treatment pathway for cleft affected children involves many different clinical disciplines, it is often protracted and complex. There is an obvious burden to the individual, but is also often a challenge for their family, both financially and emotionally (Kun *et al.,* 2013).

2. Literature Review

2.1 Incidence of CLP

The highest incidence of cleft lip and/or palate is seen in native Americans, with an occurrence of 3.6 in 1000 births, whilst the lowest incidence is seen in African Americans with 0.3 per 1000 live births (Croen *et al.,* 1998, Tolarova & Cerenka, 1998). Cleft lip and palate is more common in men (2:1), whereas isolated cleft palate is more common in women (2:1) (Tolarova, 1987). Unilateral clefts occur more commonly on the left (Bender, 2000) and on average, 70% of unilateral clefts of the lip and 85% of the bilateral clefts of the lip occur with a cleft palate (Lettieri, 1993).

Socioeconomic status is thought to have an impact on the incidence of cleft lip and/or palate, with it being lower in non-native Philippine and Chinese new-borns in the United States when compared to the incidence in the countries of origin (Murray *et al*., 1997; Croen *et al.,* 1998; Tolarova & Cerenka, 1998). Although several studies considered the effect of socioeconomic status on CLP development, be that as a result of altered environmental effects (for example, not taking vitamin supplements) or coming from an uneducated background, there is still little available evidence on the correlation of socioeconomic status and its direct effect on CLP development. One of the reasons for this is a lack of consistent definition of socioeconomic status.

2.2 Orofacial Development

Development of the lip and palate begins with migration of neural crest cells, which then influence mesenchymal tissue to form the structures of the craniofacial region. This includes

the formation of the frontonasal prominence, maxillary processes and mandibular processes.

a) Lip and primary palate development

The maxilla, notably the upper lip and alveolus is formed by the merging of the maxillary prominences with the lateral and medial nasal processes at around the 6th to 7th week of intrauterine life (Gorlin *et al.,* 2001). Development of the palate takes place during the 5th to the 12th week of intrauterine life following upper lip fusion and is divided into the primary and secondary palate. The primary palate develops from the merging of the maxillary prominences and the medial frontonasal processes, to form the philtrum of the upper lip, the alveolus and palate associated with the four upper incisors.

b) Secondary palate Development

The secondary palate, which comprises both the hard and soft palate, develops around the 6th week in utero, and is formed from the palatal shelves. Initially these shelves, which arise as extensions of the maxillary prominences, are in a vertical position either side of the tongue. However, during the 7th to 8th week they rapidly assume a horizontal position above the tongue (Moore & Persaud, 1993). Fusion of the palatal shelves begins anteriorly in the midline around the 10th week, eventually separating the oral from the nasal cavity. Following hard palate formation, soft palate and finally uvula development starts around the 12th week.

c) Development of Cleft Lip and/or Palate

The development of the cleft of the lip and/or palate is a result of the interplay between genetic and environmental influences but the exact aetiology is unknown. Whatever the principal cause, clefts of the lip occur because of a failure of the lateral and medial nasal

processes and maxillary prominence to fuse (Bender, 2000). When the epithelial tissues come into contact during fusion, a well-ordered sequence is initiated, where the epithelial seam breaks down through a combination of apoptosis, cell death and epithelial-mesenchymal transformations. Subsequently the mesenchymal tissues flow and tissue differentiation provides the framework for the structure of the face. If the epithelial seam fails to break down then the prominences cannot fuse, which results in a cleft lip either on one side only (unilateral) or on both sides (bilateral) (Rice *et al*., 2004). Failure of either maxillary migration to form the secondary palate, or failure of fusion will result in the development of a cleft palate (Sperber, 2002). Therefore, any disruption in the formation and differentiation of the lip and palate during the 4^{th} to 12^{th} week of gestation leads to the formation of cleft lip and/or palate (Sperber, 2002).

2.3 **Classification of Cleft Lip and Palate**

Cleft lip and/or palate is phenotypically diverse. Individuals can be born with cleft lip, cleft palate, or both, and it ranges from submucosal through to a complete cleft, affecting one (unilateral) or both sides of the face (bilateral) (Schutte & Murray, 1999). Clefts are usually classified as cleft lip, alveolus with or without cleft palate (CL/P) and cleft palate only as illustrated in Figure 1 (Mossey, 2009).

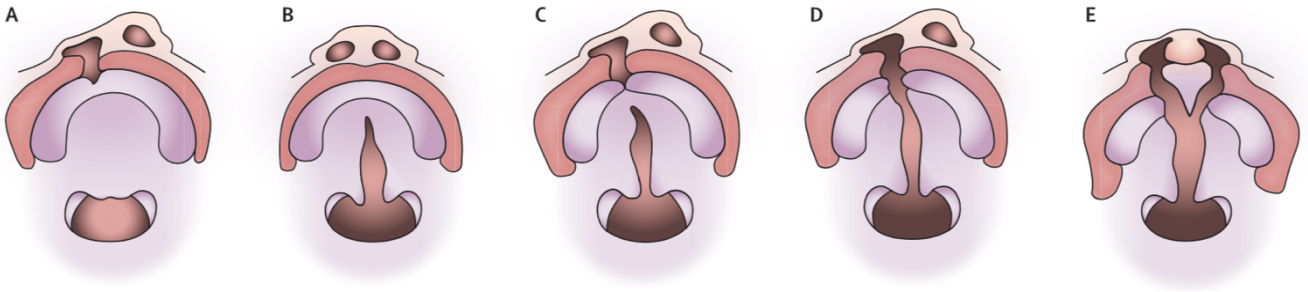

Figure 1. Nonsyndromic Orofacial clefts (Mossey, 2009): (A) Cleft Lip and alveolus (B) Cleft palate (C) Incomplete unilateral cleft lip and palate (D) Complete unilateral cleft lip and palate (E) Complete bilateral cleft lip and palate.

2.4 Aetiology

In any nonsyndromic cleft lip and palate (NSCLP) affected individual it is difficult to identify the precise aetiology because of the complex interplay of both genetic and environmental factors. If the aetiology was known it might aid both treatment and prevention.

a) Genetics

The concurrence rate for cleft lip and/or palate in monozygotic twins is 40-60% compared to 5% for dizygotic twins (Mossey and Little, 2002) suggesting a genetic component to the aetiology. However, the fact that concurrence in monozygotic twins is not 100% indicates that genetics alone is unlikely to be responsible for orofacial clefting. Chromosomal anomalies and mutations are thought to play a significant role in the development of cleft lip and/or palate. Linkage and association studies have identified the role of transforming growth factor alpha (TGFα) and Msx homeobox 1 (*MSX1*) in promoting cleft development (Shutte and Murray 1999). Whereas association studies help in the identification of potential genes involved in the aetiology of cleft

lip and/or palate, linkage studies enable researchers to classify the chromosomal segments shared between affected individuals.

b) Environmental factors

An understanding of environmental factors that cause cleft lip and/or palate might identify risk factors or help develop interventions to reduce the incidence of children born with cleft lip and/or palate. Associations between orofacial clefts and maternal environmental exposures such as smoking (Lorente *et al.,* 2000), alcohol consumption (Murray, 2002), nutrition (Krapels *et al.,* 2004a, 2004b) and maternal febrile illnesses (Natsume *et al*., 2000; Reefhuis and Cornel*,* 2002) have previously been established. There are known agents which increase the risk of developing cleft lip and/or palate which include thalidomide, dioxin and retinoic acid (Wyszynski *et al.*, 1996). There are putative links between diseases such as diabetes (Hrubec *et al.,* 2009) and hypertension with orofacial clefting (Hurst *et al.,* 1995).

A reduced level of parental educational attainment and low socioeconomic status are markers for smoking and health status of the family which might be related to orofacial clefting (Yang *et al*., 2007; Omo-Aghoja *et al.,* 2010). Conversely there are studies which show there is no association between socioeconomic status and cleft development (Carmichael *et al.,* 2003). There appears to be an effect of increasing paternal age and orofacial clefting (Green *et al*., 2010; Omo-Aghoja *et al.,* 2010) together with links between paternal occupation such as farming, painting and motor vehicle operating (Mirilas *et al.,* 2011; Desrosiers 2011).

c) Associated syndromes

It has been estimated that 5-7% of all syndromes are linked to orofacial clefting (Wong and Hagg, 2004), with over 300 syndromes resulting from a single gene defect having a relationship with cleft lip and/or palate. There are very few single gene defects linked to orofacial clefting that present with no or one additional anomaly, probably the best known being Van der Woude syndrome (mutation of the interferon regulatory factor-6 gene) and autosomal recessive ectodermal dysplasia syndrome (mutation of the poliovirus receptor related-1 gene) (Suzuki *et al.*, 2000; Kondo *et al.*, 2002). One of the most frequently occurring syndromes linked with cleft lip and/or palate is trisomy 13 (Calzolari *et al.,* 2007). Others include, Hay–Wells syndrome, DiGeorge syndrome and Treacher Collins syndrome (Gorlin *et al.*, 2001; Lindsay *et al.*, 2001; McGrath *et al.*, 2001).

Certain medications can also be responsible for syndromic effects as well as orofacial clefting. For example, hydantoin, an anticonvulsant used in the treatment of epilepsy, leads to the development of V-shaped eyebrows, cleft palate and mental disability (Hanson and Smith, 1975). Excessive alcohol consumption during pregnancy leads to foetal alcohol syndrome with facial features including midfacial growth deficiency, increased upper lip length, a thinned upper vermilion border and clefting (Jones and Smith, 1975).

2.5 Diagnosis of Cleft Lip and Palate

In the case of the unborn child, the use of prenatal ultrasound can detect foetal structural abnormalities in 80% of foetuses at the 14 week scan (Carvalho *et al.,* 2002). Clefts of the palate are more difficult to diagnose (Johnson and Sandy, 2003). There is a need for well trained staff

and the use of high-resolution ultrasound to optimise detection rates (Bender, 2000). Foetal positioning and poor resolution may obscure the diagnosis of cleft lip and/or palate when abdominal ultrasound is used. This can be overcome with the use of vaginal ultrasound (Benacerraf & Mulliken, 1993). If cleft lip and/or palate remains undetected prior to birth, a thorough physical examination of the baby's mouth, nose and palate after birth will confirm the presence or absence of cleft lip and/or palate. Initially, a submucous cleft might not be recognised as the intact epithelial lining of the mouth can mask the underlying muscular or hard tissue cleft.

Children born with cleft lip or cleft palate are normally referred to a multidisciplinary team for consultation and treatment considerations. At the first consultation it is usual to identify possible causes of the cleft lip or cleft palate and if appropriate to consider further investigation such as chromosomal testing.

2.6 Who treats orofacial clefts?

The treatment of children born with a cleft starts shortly after birth and continues usually until the early years of adulthood. A large team of health care professionals are involved in the care of individuals born with cleft lip and/or palate and include:

- Oral and Maxillofacial surgeons
- Speech and language therapists
- Clinical Psychologists
- General Dental and Medical Practitioners
- Specialist Paediatric dentists

- Orthodontists
- Prosthodontists
- ENT (Ear, Nose, Throat) surgeons
- Plastic surgeons

2.7 Problems and Treatment

There is a well-established timeline for the treatment of CLP, but this may alter according to the individual needs of the child (Figure 2).

The lip is usually repaired at around 3 months of age (around 10 weeks of age, when the baby weighs 10lbs and with a haemoglobin level of 10 mg/ml). The repair may or may not be revised in the future at the time of later surgery. Some advocate repairing clefts just after birth to utilise foetal healing, but there is no clear evidence that this is advantageous for the child and the lip repair (Schendel, 2000). A common issue faced when a child is born with CLP and before any corrective cleft lip and the cleft palate surgery, is feeding. The child is often unable to form a complete oral seal either because of the cleft lip or the cleft palate or both and there may be nasal regurgitation with liquids and solid food. Special feeding devices such as flexible feeding bottles, specialist teats or palatal obturation can also be used in the early days of a new-born before the surgery is commenced. Clefts of the palate are usually closed at 6-9 months of age.

Hearing loss and ear infections are common. The ear is compromised through abnormal anatomy and function of the Eustachian tube in patients with clefts of the palate. Children born with a cleft are therefore more prone to develop ear infections since there is poor

clearance of middle ear fluid. Prolonged and untreated ear infections lead to the development of hearing loss. It is usual to include regular hearing checks and if appropriate consider the use of grommets.

Teeth are often missing but can be ectopic or additional and will require paediatric and orthodontic intervention. There are also alveolar ridge defects which may lead to displacement of permanent teeth and prevention of their eruption. At around the age of 8-9 years and usually when the upper permanent canine root is two thirds formed, alveolar bone grafting is carried out (Lilja *et al.,* 1987; da silva Filho *et al.,* 2000). The graft has two purposes. Firstly, it is important to unite and stabilise the two maxillary segments and secondly it facilitates the eruption of the permanent canine through the graft (Bergland *et al*., 1986). Prior to grafting the orthodontist may use a fixed appliance to align the teeth and provide some arch expansion. The paediatric dentist and general dental practitioner provide preventative treatment and restorations of any hypoplastic teeth. Topical fluoride needs to be applied regularly and the use of pit and fissure sealants in patients with high risk caries is desirable to prevent caries development. Restorative care should include prevention of dental caries since tooth loss will complicate the overall treatment.

Speech issues are apparent for those born with cleft palate. Clefts of the lip only will compromise labial sounds, but as the lip is repaired by three months this is not usually a problem. Patients with cleft palate may develop nasal sounds and together with pharyngeal restrictions speech may be compromised and difficult to understand (Grunwell *et al.,* 2001).

Surgery may be needed for this, but it is the speech and language therapist who is central to correcting speech and determining the appropriate interventions (Sell *et al*., 2017).

Anxiety and depression have been reported in those born with cleft lip and/or palate (Ramstad *et al.,* 1995). This may be associated with dissatisfaction of facial appearance (Hunt *et al*., 2005) and these psychological problems are not only exhibited in patients but also their parents. These can arise when raising and caring for a child born with cleft lip and/or palate (Turner *et al.,* 1997).

Once facial growth has ceased there may be a need for orthognathic correction which will involve further surgery. One of the negative long-term effects is that most of the CLP patients have maxillary growth restriction resulting in a Class III profile, which can negatively impact self-esteem and speech. This maxillary hypoplasia is often a result of the corrective surgery carried out at a young age to correct the cleft deformity rather than a result of a genetic skeletal deformity (Kuijpers-Jagtman and Long, 2000)

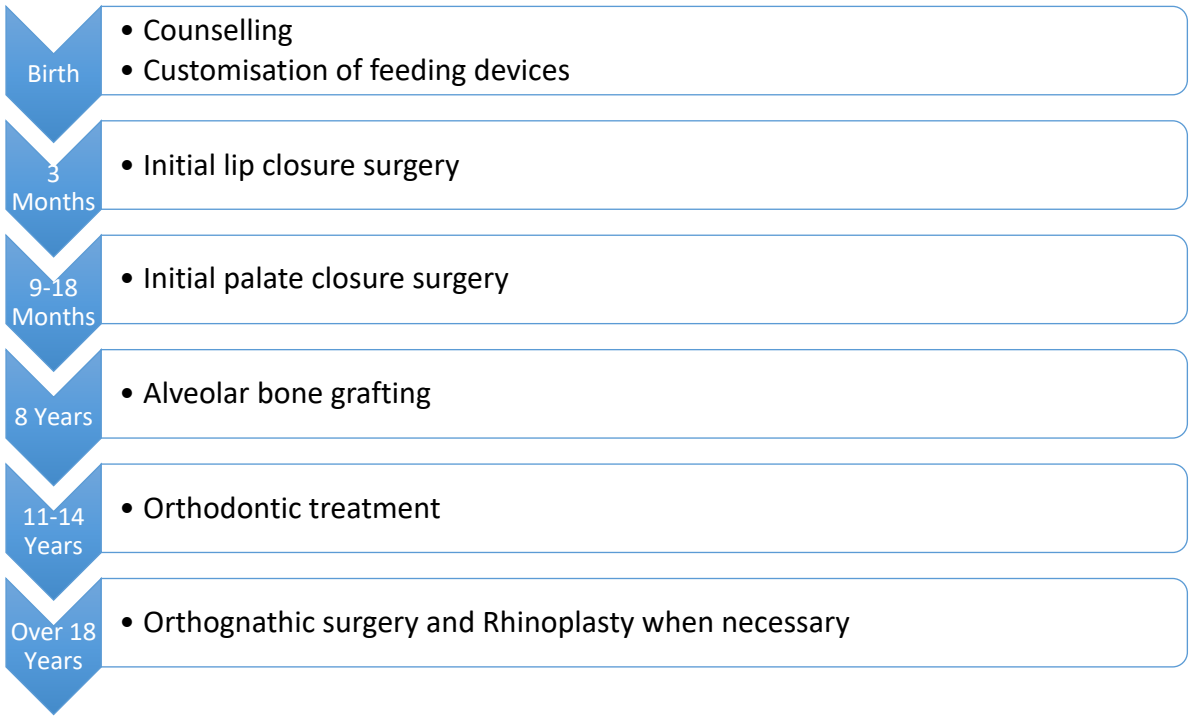

Figure 2. Classic treatment timing and approach for children with orofacial clefting (Center for Disease Control, 1995).

2.8 Preventive measures

There is some evidence that the addition of folic acid to maternal diet reduces the risk of neural tube defects such as spina bifida (Hartridge *et al.,* 1999; Wehby and Murray, 2010), but the protective effect for cleft lip and/or palate is not conclusive. The avoidance of alcohol, certain drugs (Lammer *et al.,* 1985), cessation of smoking (Zhu *et al.,* 2009), and improving overall lifestyle are also thought to reduce the risk of the development of cleft lip and palate.

2.9 Associated phenotypes with Cleft Lip and/or palate

Orofacial clefting is associated with a number of other phenotypes, which are often subclinical and more complex than just cleft lip and palate. A review by Weinberg *et al.* (2006) in

Pittsburgh concluded that phenotypic features play a role in familial transmission of orofacial clefts. This research group is actively enrolling complex orofacial cleft affected individuals and their families to help in the identification of microform features that will help with the interpretation of the interaction of genes, both with other genes, their products and environmental factors.

Compared to the general population, evidence exists that children with cleft lip and/or palate tend to have a deficiency in growth hormone and a short stature (Rudman *et al*., 1978; Lipman *et al*., 1999). However, whilst children born with cleft lip and/or palate tend to have a shorter stature than the general population, pituitary gland development appears to be normal (van der Plas *et al.,* 2012*)*. This is not without controversy and others have suggested there are functional disorders of the pituitary (Lipinski *et al*., 2010).

Other tissues affected because of clefting include adenoidal tissue enlargement (Imamura *et al.*, 2002) with a resultant decreased oropharyngeal airway volume (Celikoglu *et al*., 2014).

There is also an indication that left-handed dominance is a marker for abnormal brain lateralisation in cleft lip and/or palate. The mesoderm of the face is derived from neural crest cells and it is possible that there is a relationship between the aetiology of cleft lip and palate and the determinant of cerebral hemisphere dominance. Studies reporting left-handedness in cleft lip and/or palate patients include Wentzlaff *et al*. (1997), Daskalogiannakis *et al.* (1998), Jeffery and Boorman (2000) and Scott *et al*. (2005). It is important to note that left-handed dominance has also been associated with several conditions involving brain dysfunction including autism and schizophrenia (Dollfus *et al.,* 2002; Escalante- Mead *et al.,* 2003).

Atypical hair whorls have been associated with abnormal brain development, with the direction of hair growth determined by the pressure of the underlying tissues (Jones, 1997). Hair whorls most generally rotate clockwise, but relatives of individuals affected by cleft lip and/or palate display an increased frequency of counterclockwise hair whorls (Klar, 2003; Scott *et al*., 2005). This might suggest that these individuals are carriers of a genetic predisposition to abnormal brain lateralisation and/or clefting.

Several studies have attempted to outline the relationship between cleft lip and palate and developmental abnormalities. Functional disorders of the pituitary gland have been demonstrated to lead to developmental deficiency in individuals born with cleft lip and palate (Lipinski *et al*., 2010). There is a suggestion that the dimensions of the Sella Turcica vary in relation to pituitary function (Swallow and Osborn, 1998), and although some studies have reported deviations in the morphology of the Sella Turcica in individuals born with clefts (Nielsen *et al.,* 2005; Alkofide, 2008; Sundareswaran and Nipun, 2015; Yasa *et* al., 2017), recent work by Cesur *et al.* (2018) showed no significant difference in the measurements of the Sella Turcica.

Nopoulos and her colleagues (2000, 2002), reported significant abnormalities in brain morphology, with enlargement in the anterior region of the cerebrum and cerebellum in cleft affected individuals compared with unaffected individuals. However, the effect on the intelligence quotient (IQ) is not conclusive, with some studies reporting that children with non-syndromic cleft lip and/or palate have a lower IQ compared to controls, along with

abnormalities in language function (Richman and Eliason, 2001; Nopoulos *et al*., 2002), and others reporting limited evidence of any such differences (Conrad *et al.,* 2009).

2.10 Brain development

The development of the brain and the face is correlated in both normal and pathologic conditions (Kjaer, 1995). The first signs of the developing nervous system appear as a thickening of the neural plate on the 19th day of intrauterine life (Carlson, 2004) and arising from the embryonic tissue ectoderm. The central nervous system is divided into the following:

1) Prosencephalon (forebrain).
2) Mesencephalon (midbrain).
3) Rhombencephalon (hindbrain).
4) The future spinal cord.

Around the 21st day, these divisions further subdivide into neuromeres, with six neuromeres arising in the prosencephalon (P1-P6), one in the mesencephalon (M1) and nine in the rhombencephalon (R1-R9). The neural tube forms when the edges of the neural groove meet by lateral folding in a mechanism known as neurulation. It ends on the 26th day with the closure of both ends of the neural tube (Carlson, 2004). The anterior part of the neural tube matures to become the brain, while the posterior develops into the spinal cord and the neural crest cells from which the peripheral nervous system develops. At around the 5th week in utero, the primary vesicles split into five secondary vesicles, as shown in Figures 3.

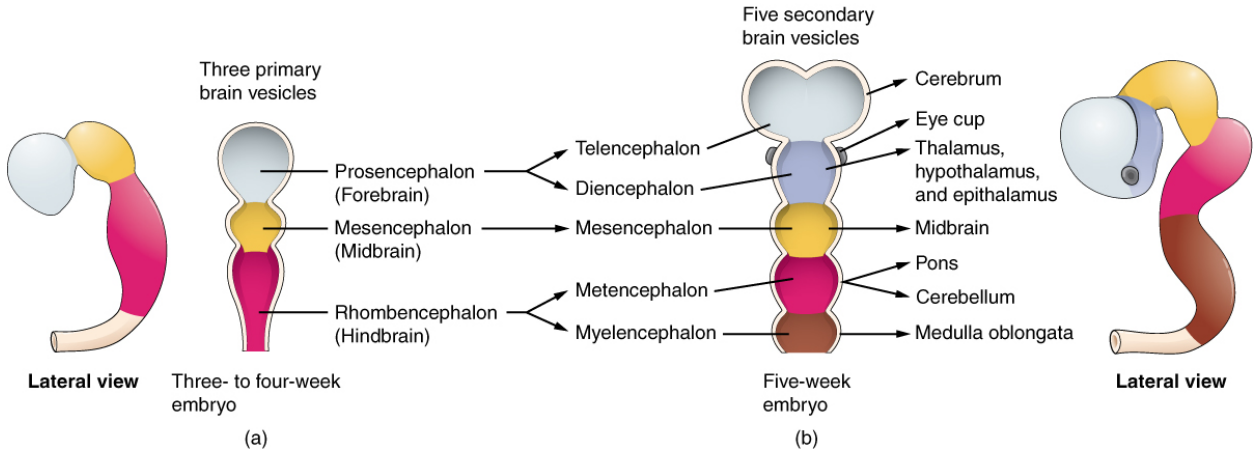

Figure 3. Embryogenesis of the brain – lateral view of the three vesicle and five vesicle stages (Openstax, 2016)

The telencephalon then expands rapidly to form the cerebral hemispheres, the outer folds (Levine and Barnes, 1999) and sulci. At around birth, most of the gyri and sulci are present, although development continues postnatally. The diencephalon ultimately gives rise to the thalamus which provides a synaptic relay centre connecting the higher brain centres to the other sections of the brain; the brainstem and hypothalamus, which offers haemostasis by influencing the behaviour in general through regulating hormone secretion. The mesencephalon progresses to become the inferior and superior colliculi and the cerebral peduncles. The former contributes to the auditory and visual system, while the latter encloses the motor nerve tract running between the brain and the spinal cord. The metencephalon develops into the pons, which serves as a channel for tracts involving the brain, the spinal cord and the cerebellum, which accounts for sensorimotor coordination and selected cognitive functions including attention, working memory, language and emotional processing (Stoodley and Schmahmann, 2009; Strick *et al.*, 2009). The myelencephalon gives rise to the medulla

oblongata. This contains centres which regulate the heartbeat and respiration, in addition to serving as a major conduit for tracts (such as the Pons) connecting the brain and spinal cord.

2.11 Development of the brain in childhood and adolescence

Ninety-five percent of the brain's adult size is reached by the age of 5 years. However, development continues throughout childhood and adolescence (Kretschmann *et al.,* 1986; Giedd *et al.*, 2009). The central nervous system develops earlier than the other tissues of the body as demonstrated in Scammon's growth curve (Figure 4). The cerebrum develops from the inferior to the superior and the posterior to the anterior (Yakovlev and Lecours, 1967). The total cerebral volume peaks around 10.5 years in girls and 14.5 years in boys, and both cerebellar volumes peak around two years later in both genders (Lenroot *et al.,* 2007; Mackie *et al*., 2007).

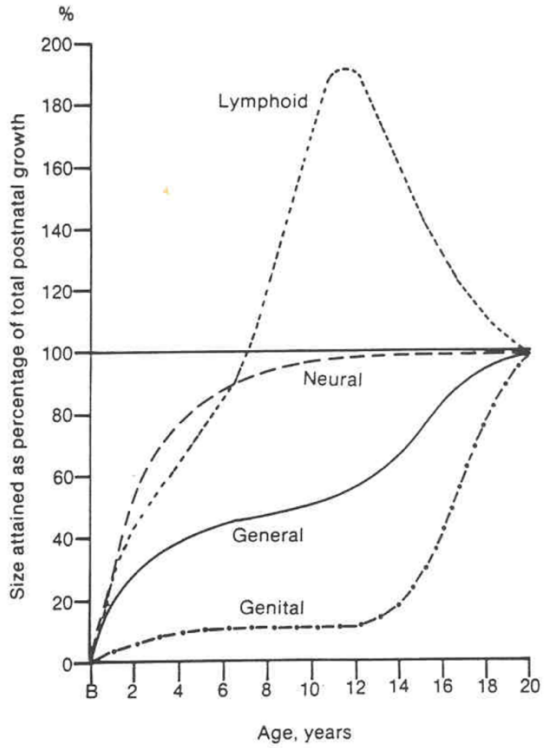

Figure 4. Scammon's curve demonstrating different tissue and organ growth (Scammon, 1930).

The grey matter volume fluctuates with time (Lenroot *et al.,* 2007; Giedd *et al.*, 2009) as a result of the materialisation and eradication of synapses and axonal myelination (Sowell *et al*., 2001). On the other hand, the white matter volume increases linearly with age and reflects ongoing axonal myelination (Sowell *et al*., 2001; Lenroot *et al.,* 2007). Although there is regional variation in the white matter trajectories, the frontal, temporal and parietal lobes have analogous trajectories. The greatest increase in the white matter volume occurs in the prefrontal cortex of the brain (Reiss *et al.,* 1996).

Unlike some other areas of the brain, the corpus callosum increases in size in the opposite direction (Thompson *et al.,* 2000), anterior to posterior, starting with the primary sensorimotor functions (anterior) and ending with higher order incorporation functions like reading (posterior).

2.11 Gender dimorphism in the brain

Both males and females have distinctive brain morphologies around birth (Gilmore *et al*., 2007). Males generally have larger brains than females from birth and throughout adulthood by approximately 10% (Sowell *et al*., 2002; Giedd *et al.,* 2009). In infancy, males have 10% additional cortical grey matter, 8% extra subcortical grey matter and finally 6% more cortical white matter.

In addition to size, brain growth trajectory is also different between females and males. The overall cerebral volume in females peaks around four years earlier than in males (Gilmore *et al*., 2007) with total grey matter peaking at around the age of 8.5 years in females 10.5 years in males (Lenroot *et al*., 2007). The same is true for grey matter volume in the parietal lobes which

peaks at 7.5 years and 9 years, and in the frontal lobes at 9.5 and 10.5 years in females and males respectively (Lenroot *et al*., 2007). Nonetheless, despite the delayed peak in trajectories found in males, their brains reveal a higher rate of change throughout childhood and adolescence in both grey and white matter (Lenroot *et al*., 2007).

However, the question remains as to whether there is any link between cognitive/ behavioural factors and the morphological differences between the genders.

2.12 Cognitive development

Neural development and cognitive development appear interrelated as the sequence of cortical maturation parallels cognitive milestones in human development (Sowell *et al*., 2004). The order of maturation occurs correspondingly, structurally and functionally, and is illustrated in Figure 5.

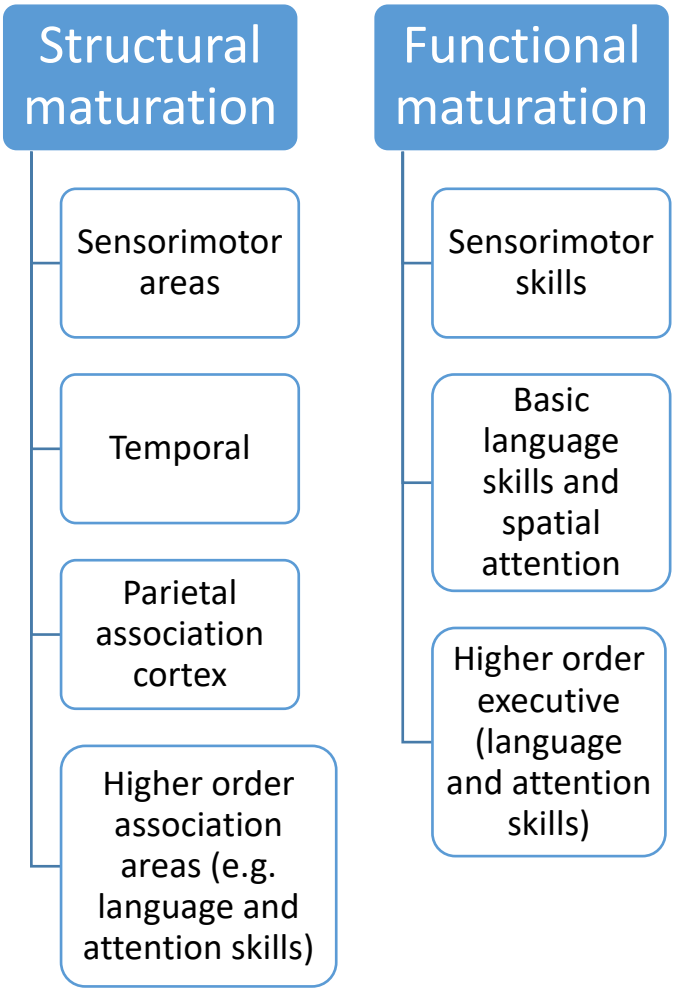

Figure 5. Structural and functional maturation order of the brain.

Volumetric proliferation in the prefrontal lobe has been found to be associated with a concurrent increase of working memory and function execution (Casey *et al.,* 1997a; Sowell *et al*., 2001). Possible evidence of a direct link between cognitive and neural development has also been suggested by Nagy *et al.* (2004) who showed, by means of diffusion tensor imaging, a correlation between working memory development and an upsurge in the prefrontal-parietal connectivity.

2.13 The interface between the brain and the face

The brain and the face are both derived from the neuroectoderm. Neural crest cells, which arise from neuromeres (neural tube segmentations), retain their distinct genetic identity when they migrate to the primordia of the budding face (Le Douarin *et al.,* 2007). The contribution of neural crest cells to the growth of both the face and brain offers further evidence that both mature together and co-vary. Fibroblast growth factor 8 (FGF8) signalling helps neural crest cells regulate the size and growth of the evolving brain. Not only do the brain and face grow from the same cells, but they also link physically by their proximity.

The anterior neural tube serves as a framework for the facial eminences (Muenke and Cohen, 2000). When mice were genetically modified to have reduced brain growth, their faces developed earlier and were more prognathic throughout embryogenesis (Boughner *et al.,* 2008), suggesting the size of the neural scaffold influences facial morphology.

The presence of facial and brain dysmorphology is well documented in various disorders, including foetal alcohol syndrome, Downs syndrome, median cleft syndrome and most notably, holoprosencephaly (Gorlin *et al*., 2001). Furthermore, autism has been associated with frontopolar brain asymmetry in the peri-orbital region of the face (Hammond *et al*., 2008).

Several signalling molecules link brain and facial growth during development. Sonic hedgehog (SHH) is a signalling molecule which is actively expressed in the forebrain, where it plays a crucial role in its patterning and in stimulating the frontonasal ectodermal zone (Schneider *et al.,* 2001; Hu and Marcucio, 2009). Blockage of SHH in the forebrain causes disruption in the signalling present in the frontonasal ectodermal zone, resulting in severe malformation in the

development of the middle and upper face along with the forebrain (Hu and Marcucio, 2009). Equally, a reduction in SHH signalling often results in medial rotation of the maxilla, hypotelorism and constriction of the frontonasal prominence. On the other hand, an increase in the SHH signalling often causes lateral divergence of the maxilla, midfacial widening and lastly frontonasal hypoplasia (Young *et al*., 2010). Facial development is therefore stalled if there is a disturbance both in the signalling molecules and physical constraints in the dysplastic scaffold and vice versa.

2.14 Cognition and cleft lip and palate

As a result of the likely close association between the development of the brain and the face, cognitive studies have suggested that individuals with cleft lip and/or palate have a greater risk of cognitive deficits. For some time, it has been recognised that children born with clefts, face potentially greater academic challenges than their unaffected peers (Richman, 1976). When individuals born with a cleft underwent standardised national achievement tests in two centres in the USA, 47% performed below the 25th percentile (Broder *et al.*, 1998). It has also been reported that fewer cleft affected teenagers attended college and even fewer gained employment in later years (Peter *et al*, 1975).

There has been some debate as to whether poor academic achievement was a result of cognitive deficits or environmental factors. These environmental factors include lower teacher and parent expectation, depression, and low self-esteem. However, there is little evidence linking these environmental factors to the cognitive function of individuals with cleft (Conrad *et al.,* 2009).

While some studies found lower IQ levels in cleft lip and/or palate patients (Estes and Morris, 1970; Nopoulos *et al.,* 2002), others have found no evidence of lower IQ (Conrad *et al.,* 2009), although lower verbal IQ was reported for children with cleft palate when compared to the controls.

Shortfalls in verbal labelling, verbal fluency, verbal memory, and visual memory have been reported in cleft affected children (Broder *et al.,* 1998; Richman *et al.,* 2005; Conrad *et al.,* 2009). Verbal deficits seem to vary by age, gender, and the cleft type among subjects with clefts (Richman, 1980). Around the age of six years, 49% of children born with cleft lip and palate and 53% of children with cleft palate have some degree of reading disability (Richman *et al.,* 1988). These figures reduce with the age of the child to 9% and 33% respectively, which for cleft lip and palate is similar to the figure of 10% of children in the general population with some degree of reading disability (Fluss *et al.,* 2008).

There are also known language deficits in children with CLP, which are similar to the pattern of language shortfalls found in children with developmental dyslexia (Richman *et al.,* 1988; Eckert, 2004; Galaburda *et al.,* 2008). Developmental dyslexia is defined as a specific learning disability categorised by discrepancies in word recognition despite normal intelligence, satisfactory educational opportunities and an absence of neuropsychiatric illness (Eckert, 2004). These language deficits in people with developmental dyslexia have been linked to structural brain abnormalities (i.e. decreased grey matter volume in the pars triangularis) (Eckert *et al.,* 2003). The similarities between language deficits observed in patients with CLP and children with developmental dyslexia implies both might be associated with structural brain abnormalities.

2.15 Brain structure in cleft lip and/or palate affected individuals

People with language disorders, but without cleft lip and/or palate, have issues in rapid verbal labelling, verbal fluency and verbal memory, which may be linked to structural abnormalities in the prefrontal cortex (Gabrielli *et al.,* 1998). As previously described, individuals with developmental dyslexia have structural brain abnormalities, notably volumetric anomalies in the inferior frontal gyrus, superior temporal plane and cerebellum (Eckert *et al*., 2003; Eckert, 2004). The similarity in the language deficits linked to structural brain irregularities seen in developmental dyslexia raises the likelihood of patients with CLP also having such brain irregularities.

Until recently few of the studies have specifically investigated the brain morphology of children with cleft lip and/or palate. Using magnetic resonance imaging (MRI), Nopoulos *et al.* (2007) established that brain volume was smaller in children with cleft lip and/or palate than in age/gender matched controls. The smaller brain size was a consequence of a reduction of both cerebral and cerebellar components of the brain. In 2010, Van der Plas *et al*. reassessed the same sample of children to investigate the effect of cleft side on brain volume in boys with unilateral CLP. They discovered that boys with right sided clefts had smaller total white matter (cerebral and cerebellar) volumes than boys with left sided clefts or age matched controls, but larger temporal grey matter.

Regional analysis of the ventral frontal cortex (which consists of the straight gyrus and orbitofrontal cortex) was conducted in boys with CLP by Boes *et al.* (2007). They found that there was smaller volume of the straight gyrus in children with CLP, but there was no difference

in the volume of the orbitofrontal cortex. This tissue deficit in the straight gyrus is correlated with impaired social function, which might possibly explain the shyness noted in individuals with CLP (Bressmann *et al.,* 1999; Boes *et al*; 2007).

Using the same sample of males from the University of Iowa, five studies were conducted on the brain structure of adults in non-syndromic cleft lip and/or palate. Nopoulos *et al.* (2000b, 2002a) found a significantly smaller cerebellum size in adults with cleft lip and/or palate. Furthermore, in the cerebrum they discovered that the frontal and parietal lobes were larger in these patients, while the temporal and occipital were smaller.

In addition, Weinberg *et al.* (2009) recognised lateral displacement of the frontal and occipital poles, an increase in the span of the cerebellum and a posterior displacement of the corpus callosum in these patients. Furthermore, an increase in the size of the superior temporal plane and the left planum temporale was noted, both of which link to cognitive deficits when abnormal (Shriver *et al.,* 2006).

Two studies conducted by Goldsberry *et al.* (2006) and Becker *et al.* (2008) used positron emission tomography (PET) on CLP patients instead of MRI. They observed hyperactivation of the right cerebellum and left frontal operculum during simple language tasks, and hypoactivation of inferior parietal lobule and Wernicke's area during complex language undertakings. Furthermore, there was a reduced blood oxygen level supported response in the lateral prefrontal cortex, superior and middle temporal gyri during word generation tasks. These results add to the existing evidence that would seem to suggest the presence of brain abnormalities in CLP patients.

Although further investigations into brain structure are required, the systematic review conducted as part of this research will help summarise and evaluate the existing published data for cleft lip and/or palate patients.

2.16 What is a systematic review and a meta-analysis?

A systematic review aims to recognise, evaluate and integrate all the available research papers on a topic, using methods that fit pre-specified criteria (Armstrong *et al.,* 2011). The aim is to answer a specific research question following the review and any potential meta-analysis, using a structured methodology to minimise bias. Systematic reviews should be objective, ordered, and iterative. After collecting the results of the search, where possible a meta-analysis can be performed to summarise the results of all the previous research papers identified in the systematic review. The following is a simplified definition from Glass (1976) who first described a meta-analysis to be "statistical analysis of a large collection of analysis results from individual studies for the purpose of integrating the findings".

2.17 Hierarchy of Evidence

It is key to consider the level of evidence available when conducting the search. The diagram below (Figure 6) indicates that the highest level of evidence is the Meta-Analysis located at the top of the pyramid, and with the lowest being expert opinion (Haynes *et al.,* 1997). An example of a good systematic review is one carried out by Harrison *et al.* (2007) where they compared the effectiveness of orthodontic treatment in early age and adolescent patients who had prominent upper teeth. They performed a quality analysis and a statistical analysis accounting for both dichotomous and continuous outcomes in the studies included in their review. The

conclusion suggests that early treatment followed by a phase of later treatment showed no advantage over a one-phase treatment. The methodology used provides strong evidence for this conclusion. However, in some cases the only evidence available is expert opinion, and this limits the implementation of any identified outcome of the review by subsequent healthcare professionals.

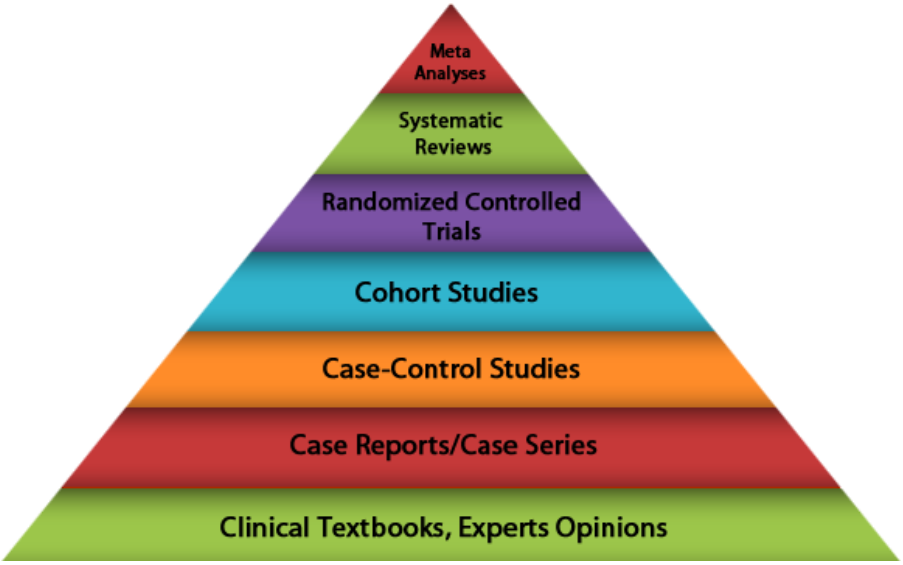

Figure 6. Level of evidence hierarchy (Sackett, 2000).

2.18 Developing a systematic review

Systematic reviews are structured with clear objectives which comprise (Khan *et al*., 2003):

1) Identification: All related published and unpublished evidences should be collected to answer the intended healthcare question/s. Selection bias, language bias and publication bias should be avoided.
2) Selection: Include subjects relevant to the research question by assembly of objectives, interventions and outcomes of interest in selecting studies relative to the review.

3) Appraise: The quality of each study should be assessed. Poor quality research is excluded after discussing the reasons why it was eliminated.
4) Combine: Individual findings from different studies are usually integrated without bias. Conclusion of the clinical effectiveness and convenience is then outlined in the systematic review.
5) Summarise: Results should be gathered and any weaknesses in the findings should be taken into consideration.

2.19 Initiating a meta-analysis

When all data is assembled, all appropriate summary measures are usually calculated to develop an "Effect size" measure. Effect size measures signify the different average scores between intervention and control groups. Standardisation is key between studies as units of measurements across studies vary, and in order to produce an effect size estimate all measurements need to be standardised.

Some studies carry results that deliver more weight than others. This means that large studies often have a greater influence on the results of a meta-analysis because of their larger sample sizes.

When analysing the results, it is important to select an appropriate statistical model, and there are two main choices, fixed effects or random effects. The fixed effects model "assumes that each study is evaluating a common treatment effect", while the random effects model "assumes that the treatment effect in each individual study is different across each study" (Higgens, 2011). The latter model attempts to account for additional variation in the effect size, which is often assessed in a meta-analysis through "heterogeneity". When presenting the

results of a meta-analysis the most frequently used summary presentation is the 'forest plot'. The boxes for each study highlight the studies with the greatest weights, as each box is proportional to its effect on the overall outcome. The summary estimate of all the studies is the summary diamond, which is usually found at the bottom of the forest plot. The confidence interval is given by the width of the summary diamond. Of interest is the position of the summary diamond and whether it crosses the line of no effect within the plot.

2.20 Importance of systematic reviews and meta-analysis

Professionals working in healthcare require updated and evidence-based information on effective and appropriate medical interventions. Systematic reviews help to deliver information in a straightforward manner, reducing the time required for the general reader to search for such data. Gaps in research are commonly highlighted in systematic reviews and future research proposals are usually suggested to help narrow the evidence gap. In the past, certain healthcare practices have been classified as "experience based" or "habit based" (Law, 2002). However, systematic reviews are associated with "recent evidence-based practice", which aims to eliminate bias and focus on extracting interventional methods to deliver the best possible care to patients.

2.21 Limitations of systematic reviews

Even though systematic reviews feature in the hierarchy of evidence, systematic reviews can vary in quality, which can in turn result in weaker or incorrect information being published. Caution must therefore be exercised when basing decisions on a systematic review. Some of

the questions that can help identify weak systematic reviews (Crombie and Harvey, 1997) include:

- Is the topic clear-cut?
- Is the search strategy described?
- Are the inclusion and exclusion criteria fairly applied?
- Do the included studies have similar effects?
- Was the play of chance assessed?
- Is the recommendation based on quality of service present?

If most of these questions are well answered, then an estimate can be made regarding the strength of the systematic review.

With respect to neuroanatomy, in particular brain structure, brain development and any potential links to the development of CLP might display underperformance (Richman and Eliason, 2001; Nopoulos *et al*., 2002). Although a number of studies have been published on different aspects of the brain and CLP, the evidence for any associations between the two are not are not always clear cut. Therefore, this current research specifically focused on brain structure and its alteration in cleft lip and/or palate affected individuals.

3. Methods

3.1 Aims

The aim of this systematic review was to determine if there is evidence for a relationship between the presence of a non-syndromic cleft lip and/or palate and altered brain structure/function in cleft affected individuals.

3.2 Objectives

- To explore the evidence available reporting on the possible relationship between the presence of a non-syndromic cleft lip and/or palate and altered brain structure/function.
- To identify the types of altered brain structure/function that are reportedly associated with cleft lip and or palate.
- Where such a relationship or otherwise exists and where supported with summary data, the evidence would be included in a meta-analysis.

3.3 Literature search

An electronic database search was conducted on three platforms: Ovid (MEDLINE), Ovid (EMBASE) and the Cochrane library. The search was limited to English language only and included publications from 1st January 1969 until February 8th, 2019. An additional manual search was done on reference lists of the identified papers in order to add further research that might have been missed in the electronic search.

3.3.1 Search strategy

With the help of a librarian from The University of Bristol, the following keywords where used in Ovid (MEDLINE & EMBASE) and Cochrane search:

1) *Cleft Lip/*
2) *Cleft Palate/*
3) *("cleft lip" or "cleft lips" or "cleft palate" or "cleft palates" or "orofacial cleft").mp.*
4) *1 or 2 or 3*
5) *Phenotype/*
6) *Phenotype*.mp.*
7) *5 or 6*
8) *4 and 7*
9) *exp BRAIN/*
10) *("IQ" or "brain*").mp.*
11) *9 or 10*
12) *4 and 11*
13) *7 and 11*
14) *4 and 13*

3.3.2 Eligibility criteria

The predetermined eligibility criteria for the search are illustrated in Table 1. In order to develop a research question, before a systematic review search was created, PECO (Population, Exposure, Comparator, Outcome) was implemented (Morgan *et al.,* 2018).

Inclusion Criteria	Exclusion Criteria
• Participants: Individuals born with cleft lip and palate, live births, any ethnic group, both genders, without any other associated syndromes, in hospital or community settings.	• Individuals who were still born.
• Exposure: assessment of brain structure in patients with NSCLP.	• Individuals with associated syndromes.
• Comparators: Healthy individuals.	• Non-English language articles.
• Outcome: Measure if there is presence of difference in brain structure between the cleft lip and palate and healthy individuals and if there is any alteration in the quality of life as a result of the effect.	

Table 1: Details of inclusion/exclusion criteria.

A total of 183 articles were identified in the literature search, and following deduplication the final number of articles selected was 115. The titles and abstracts of these articles were initially screened, following which, those that fulfilled the eligibility criteria were chosen for full text screening. This resulted in 15 articles fulfilling the criteria. From these 15, one article was excluded as it was a review, which left a total number of 14 articles for data extraction. Two reviewers evaluated the validity of the articles: Nadine Homoud (NH) and Zainab Al-Saffar (ZA). Any disagreement found in inclusion of the articles were then resolved by a third reviewer; Professor Anthony Ireland (AI).

3.4 Data extraction

Data was extracted using a data extraction form (See Appendix A) and included:

a) Name of author

b) Study details:

- Date of study
- Title of study
- Aim of study

c) Study design:

- Allocation of participants
- Number of participants included

d) Population/ Participants:

- Origin
- Gender
- Age
- Cleft group (type of cleft, size of sample) / Control group (size of sample)
- Setting (where patients were recruited from)

e) Exposure: type of intervention used to compare

f) Outcome measures

g) Results from analysis between groups

3.5 Risk of bias assessment

Using the Critical Appraisal Risks Programme (CASP) tool (See Appendix B), the papers were also examined for risk of bias using the following criteria:

a) Validity of the results

b) Nature of the results

c) Effect of the results locally (i.e. is it helpful?)

This led to a further five articles being eliminated as the results were not clearly outlined.

Using the remaining nine papers it was possible to identify the following subgroups based on brain structure:

- Total cerebellar volume
- Cortical grey matter
- Total cerebral volume
- Intracranial volume
- Frontal cortex and straight gyrus

Of the final nine articles, two articles were excluded from the final data analysis. This was because one included only the mean corrected volume after regression, and only reported mean differences rather than the mean value and either the standard deviation or 95% confidence interval of the mean, while the other had missing data. The authors of these papers were contacted concerning the availability of the raw or summary data, but the response was that these were no longer available.

Therefore, the following seven studies were included in the data analysis for each or some of the subgroups for brain structure (Table 2).

Total cerebellar volume	Cortical grey matter	Total cerebral volume	Intracranial volume	Frontal cortex and straight gyrus
Nopoulos *et al.,* 2007	Nopoulos *et al.,* 2002	Nopoulos *et al.,* 2007	Nopoulos *et al.,* 2007	Boes *et al.,* 2007
Nopoulos *et al.,* 2002		Nopoulos *et al.,* 2002	Nopoulos *et al.,* 2002	Nopoulos *et al.,* 2005
Devolder *et al.,* 2013		Nopoulos *et al.,* 2000	Nopoulos *et al.,* 2000	
Nopoulos *et al.,* 2000				
Conrad *et al.,* 2010				

Table 2: The seven papers included for quantitative synthesis.

The final number of articles and selection process can be seen in Figure 7 of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher *et al.,* 2009).

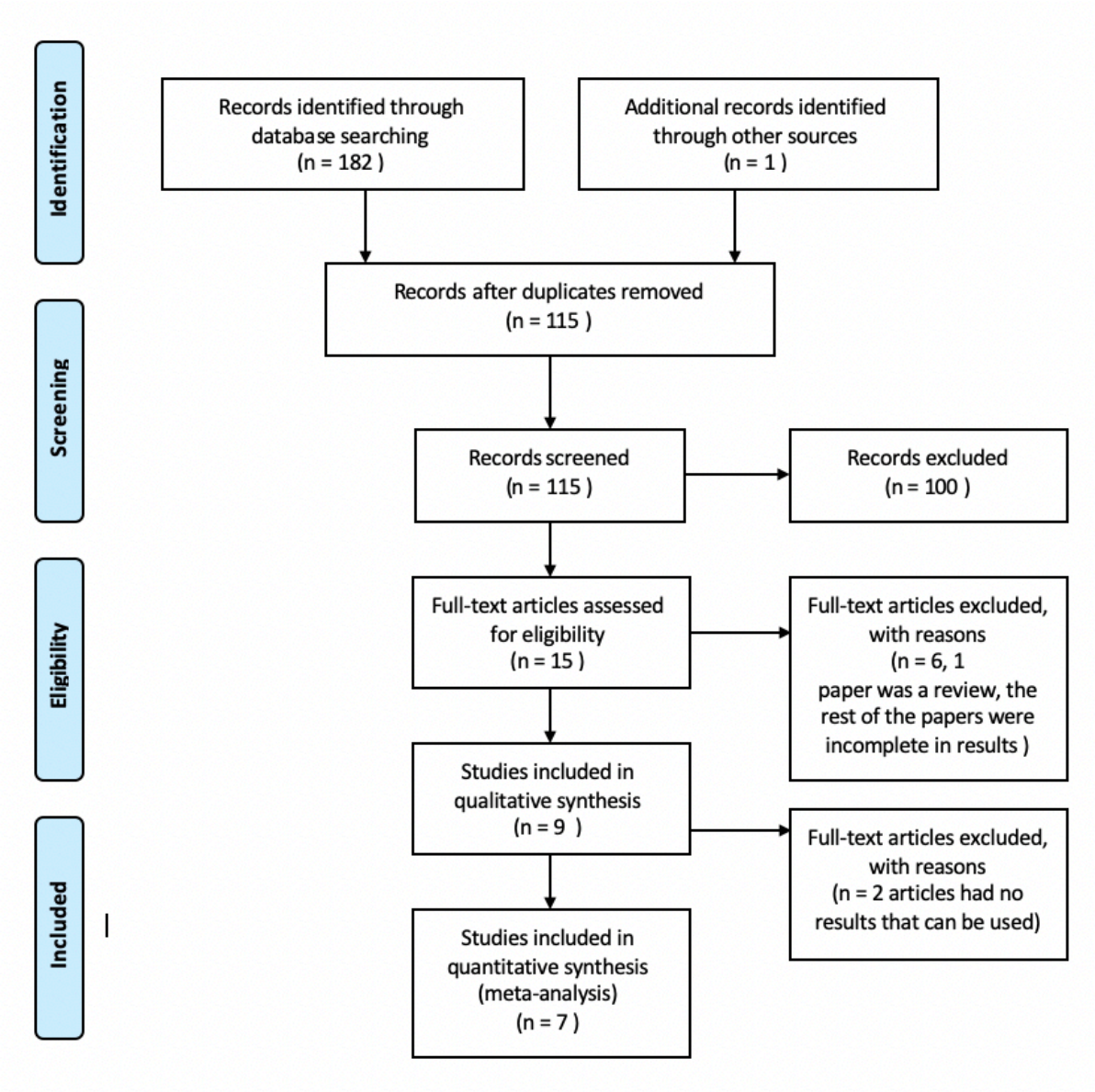

Figure 7. PRISMA flow diagram showing the article selection and reasons to eliminate papers.

3.6 Meta-analysis

A random effects meta-analysis was conducted using STATA version 16 (Stata Corp, College Station, Texas, USA) to estimate the effect of cleft lip and/or palate on the brain structure from the selected articles. In order to be included in the data analysis the papers needed to provide; sample size, mean, standard deviation, standard error or upper and lower 95% confidence intervals of the mean. The random effects meta-analysis ensures less influence of larger studies on summary approximations. For the analysis, seven papers were included (Table 2). The data were analysed by creating subgroups of different brain structures and also as the overall effect of brain structure on from cleft.

4. Results

4.1 Results of the systematic review

One hundred and eighty-three articles were initially identified in the literature search using both online search engines and hand searching. Following deduplication, the titles and abstracts of 115 articles were then screened to determine whether they fulfilled the eligibility criteria prior to full text screening. Of these, 15 fulfilled the inclusion criteria. A further article was excluded as it was a review, leaving a total number of 14 articles for full text screening and data extraction (See Figure 7). Following data extraction and a risk of bias assessment, seven papers were included for data analysis (see Table 2). A summary of the characteristics of the papers included in the systematic review (nine papers) is presented in Table 3. The data extracted from each study and the subgroupings determined in this analysis according to specific outcomes are shown in Table 4.

4.2 Reporting on the studies included

Almost all studies selected where carried out in University of Iowa, USA. All papers were case–control studies and the main examination was carried out using Magnetic Resonance Imaging to assess brain structure. Some of the papers included cognitive assessments. There was commonality in that all the papers demonstrated a difference in the morphological brain structure between controls and CLP patients. A more detailed description of each of the nine studies is given in the following section and they have been organised in chronological publication order.

4.2.1 Nopoulos *et al.,* 2000

The purpose of this study was to establish whether adult males born with cleft lip and palate had atypical cerebral morphology. Fourteen men born with CLP were recruited from the cleft registry in the University of Iowa. The CLP participant group were subdivided into three subgroups; five had incomplete bilateral cleft palate only, one participant had left cleft lip only and eight participants with cleft lip and palate, out of which one was bilateral and the rest unilateral. Fourteen controls were matched to subjects by age, gender and parental socioeconomic status in order to reduce the effects of differences on brain growth. The mean age was 33.7 years in the CLP group and 33.1 years in the control group. Images were obtained of all subjects using MRI and the image processing was performed using Brain research: Analysis of Images, Network and Systems programme (BRAINS). There were no statistical differences between subjects and controls in the intracranial brain volume, total brain tissue and the total volume of cerebrospinal fluid (CSF). However, there was a statistical difference in the volume of the cerebellum. Those born with CLP had a significantly smaller cerebellar size (P=0.04), a significantly larger frontal lobe (P=0.02) and a significantly smaller temporal and occipital lobe (P=0.02, P=0.009 respectively). The authors highlight that there is a complex interaction between craniofacial and cerebral development, but the relationship is unclear. Whether facial clefting is a consequence of a primary problem in facial growth, a primary problem in brain growth, or a result of both is not known. As there was insufficient data further studies were recommended.

4.2.2 Nopoulos *et al.,* 2002

This case control study included 92 participants, which were split into two groups: 46 subjects and 46 controls. All participants were Caucasian and their IQ's were assessed using cognitive testing. Of the 46 subjects, 14 had CPO and 32 had CLP (11 with bilateral cleft lip and palate, 18 with left unilateral cleft lip and palate and three with right unilateral cleft lip and palate). The control group was matched to the cleft subjects by gender, age (mean 30.1 years vs 28.8 years), parental SES and level of education. After quantitative measurements of the brain were obtained with MRI, images were processed using BRAINS, and the brain tissue measures were analysed using a general linear model procedure. Interestingly, there was a difference between the controls and those born with a cleft. It was noted that irrespective of phenotype, the cleft cases had an abnormally enlarged anterior region of the cerebrum, and decreased volumes of posterior cerebrum and cerebellum. Furthermore, the largest severely affected region was the left temporal lobe. A Spearman's correlation test was carried out to decrease the influence of outliers in the calculation of cerebral region volume and IQ where total brain tissue volume was controlled. The results indicated that anterior cerebral enlargements were pathologic, similar to that seen in autism and neurofibromatosis, where it showed a significant inverse correlation with Full Scale IQ (Piven *et al*., 1995; Moore *et al*., 2000). This was interpreted such that the structural abnormalities were concomitant with cognitive dysfunction.

4.2.3 Nopoulos *et al.,* 2005

It is known that the frontal lobe of the brain is linked with social function. Previous studies on NSCLP affected males have shown abnormalities in the structure of the frontal lobe. The aim of

this study was to evaluate the Ventral Frontal Cortex (VFC), a subregion of the frontal lobe, and to see if there was any association with the hypothetical social inhibition experienced in males with NSCLP. Using the data within the registry of CLP in the University of Iowa, a sample of 46 men born with cleft and over the age of 18 were contacted and invited to participate in the study. There were 14 CPO subjects and 32 CLP subjects included, of which 11 had bilateral cleft lip and palate, 18 had left unilateral cleft lip and palate and three had right unilateral cleft lip and palate. The controls were matched by age (30.1 years vs 28.8 years), gender and socioeconomic status of the family. All subjects were Caucasian. The first part of this study measured social functioning using a standardised scale from the *Psychiatric Symptom You Currently Have* (PSYCH) assessment (Andreasen, 1987). This measures recreational interests and activities, relationships with friends and peers, and relationships with family members. The second part of the study involved an MRI of the ventral frontal cortex, namely the orbitofrontal cortex (OFC) and the straight gyrus (SG), followed by analysis using BRAINS. The study showed that this patient group had substantially smaller orbitofrontal cortex volumes compared with healthy controls, which correlated with the increased social dysfunction. The straight gyrus was not found to be morphologically abnormal. However, a significant limitation of this study was that social function measures were not obtained for the controls.

4.2.4 Boes *et al.,* 2007

Similar to the Nopoulos *et al.* (2005) publication, this study attempted to determine whether there was any relationship between social function in NSCLP affected children with respect to measures of VFC morphology and self-concept. The subjects comprised 30 NSCLP boys aged

between seven and twelve years of age. The NSCLP group included eight cleft lip patients, fifteen cleft lip and palate patients and seven cleft palate patients. The comparison group were recruited through local advertising, and 43 healthy controls were selected and matched by gender and age to study group. Social function and self-concept were assessed using questionnaires, with a standardised scoring system, and these were completed by the boys and one of their parents. The cortical volume and surface area of the VFC were then evaluated using structural MRI. The results showed that NSCLP affected boys have a significantly impaired social function relative to the control group, although there was no difference in self-concept. Unlike the results reported by Nopoulos and her colleagues (2005), the MRI demonstrated a decreased volume and surface area in the left straight gyrus of the NSCLP boys. Examination of the VFC morphology revealed a significant correlation with social dysfunction, but not with self-concept. The authors advised caution when interpreting findings based on the complexity of neurobiological social behaviour and the uncertain role of VFC in normal and abnormal social behaviour.

4.2.5 Nopoulos *et al.,* 2007

This study was conducted in a tertiary care centre and was designed to determine the brain structure in 74 cleft affected individuals and 74 healthy controls. The cleft phenotypes were cleft lip only (n=18), cleft and palate only (n=33) and cleft palate only (n=23). The participant's age ranged from seven to seventeen years and the controls were matched for age and gender. Since previous studies were conducted only on adult men, this study intended to assess the differences found in both genders and the potential developmental processes involved in

growth and brain structural abnormalities. General measures of height and head circumference were obtained, and brain structure was assessed using MRI, providing general and regional brain measurements. These were analysed using covariate-adjusted multivariate analysis of variance (MANOVA). The results showed that height was significantly lower in the nonsyndromic cleft lip and palate group (P=0.03). The same children also had smaller brains, with both cerebrum and cerebellum volumes appearing to be reduced (P=0.04, P<0.001 respectively). Within the cerebrum, the frontal lobe appeared reduced (P=0.008) and the tissue distribution of cortical grey matter and white matter within the cerebrum were abnormal in boys with NSCLP, but proportional to the controls in girls with NSCLP. Moreover, children with NSCLP displayed smaller brain volume in the frontal lobe and subcortical grey matter. Meanwhile in men, total brain and cerebrum volumes were normal. The variability between the abnormal brain structure found in children with NSCLP and adults with NSCLP may suggest that the brain growth and the development trajectory is aberrant in subjects with NSCLP. Consequently, the authors suggest that a longitudinal assessment of both girls and boys with NSCLP would be important in understanding the pattern of brain growth and development.

4.2.6 Conrad *et al.,* 2010

The objective of this study was to determine if there were cerebellar structural differences in boys and girls born with NSCLP and to establish whether these differences (if present) were linked to speech impairment. Between 2003 and 2007, measures of the cerebellar volume were obtained on 43 children with NSCLP and 43 healthy controls. The phenotypes of the clefts included were seven cleft lip only, 11 cleft palate only and 25 cleft lip and palate. The NSCLP

group and control group had an average age match of 13.27 years and 13.28 years. In total there were 72 boys and 57 girls. The children were screened for medical, psychiatric, speech/language and behavioural concerns and children with NSCLP received detailed speech evaluations. Similar to the Nopoulos *et al*. (2007) study, the results showed that boys with NSCLP had a smaller cerebellum volume than controls (p=0.002), whereas in girls, only regional reductions in size were significant (corpus medullare p=0.04). In boys the cerebellum size correlated with articulation (p=0.045). There were admitted limitations to the study. This was mainly in the screening process and recruitment of the control subjects, where some of the children with learning, speech and health concerns were included. Likewise, there was a lack of variability in the speech measures, which may have curbed the power to detect correlations with the cerebellum's effect on speech, as it might have been linked to other developmental syndromes. Lastly, the influence of the cerebellum on speech may have been related to different aspects of speech that were not assessed in the study. They concluded that a larger sample of children with a greater spectrum of speech difficulties (ranging from excellent to very poor) is required for future studies. It will also be important to try and determine to what degree the speech deficits may have been due to abnormal oral structure and oral function in children with clefts, rather than structural abnormalities of the brain.

4.2.7 Van der Plas *et al.,* 2010

This study recruited 14 boys with right sided CLP and 19 boys with left sided CLP. The healthy controls were 57 boys matched for age ranging from seven to seventeen years. The research sought to determine whether the side of the cleft is in any way related to brain structure. The

findings showed that the total white matter was significantly reduced in boys with right sided clefts, compared with left sided clefts and unaffected healthy controls. Furthermore, regional analyses demonstrated that reductions in white matter were evident in both the cerebellum and the cerebrum in boys with right sided clefts. In the case of the cerebrum, the white matter volume was lower in both the frontal lobes and occipital lobes. From these early results, it would seem that laterality, namely a right sided cleft, is possibly associated with more abnormalities in brain structure.

4.2.8 Devolder *et al.,* 2013

A total of 234 participants were included in this study, which was the largest of the studies included in this review. Here, the cerebellar structure within two primary subtypes of NSCLP, namely CL/P and CPO, was assessed. One hundred and seven subjects, separated by gender, were compared to 127 healthy controls. Brain structures were compared between the groups using MRI. The results showed that males had significantly lower cerebellum volumes in the NSCLP group (P=0.001) compared to controls. Regionally within the cerebellum, males with NSCLP had a larger anterior lobe and a smaller superior posterior lobe (p=0.047, p=0.019 respectively). CPO males showed only some regional changes compared to controls, with no reduction in the overall volume. By contrast, females with NSCLP displayed no overall cerebellar abnormalities compared to controls, although females with CPO displayed significantly lower cerebellum volumes when compared to controls. The results demonstrate that abnormal cerebellar morphologies are dependent on cleft subtype as well as gender, adding to the body of evidence that CLP and CPO are separate entities. A limitation of this study was the small

sample size with respect to the CPO group, as the prevalence of CPO in a population is relatively low. A larger sample size might have addressed this issue. Another limitation was that participants were excluded if they had low IQ (below 70). This might have prevented the enrolment of participants with NSCLP that may have perhaps had more marked structural abnormalities possibly related to their clefting, or not.

4.2.9 Adamson *et al.,* 2014

This Australian case control study recruited 52 individuals; 26 NSCLP affected individuals identified from the Cleft Registry Database at the Royal Children's Hospital in Melbourne, and 26 unaffected controls matched by age (ranging from 6 to 14 years old) and demographics. The number of males was slightly higher than females in the total sample. Using high resolution MRI, volumetric analyses of the brain with respect to both regional cortical volume and thickness were obtained. The results showed abnormally large cerebral cortex grey matter volumes with decreased volumes of subcortical grey matter and cerebral white matter. This study was the first to report abnormal cortical thickness in NSCLP affected individuals. The findings suggest that overall, the brains of children with NSCLP are less mature than those of their age-matched peers. The gender specific comparisons also revealed that NSCLP females were more immature compared to their non-cleft peers and when compared to NSCLP males. This research was unique in comparison to other similar studies in that it employed a more detailed segmentation technique (FreeSurfer), which allowed for a more fine-grained morphological analysis. Earlier studies focused on specific regions of interest, namely the OFC

and straight gyrus, and employed manual and automated segmentation techniques respectively (Nopoulos *et al*., 2005).

4.3 Participants

895 individuals participated in the nine studies described, of which 419 had cleft. The age of the participants ranged from approximately six years to thirty years. The total number of males who participated in the studies was 680 and there were 215 females. The socioeconomic status was accounted for in most of the studies except for one (Adamson *et al.,* 2014), in order to minimise external factors that might affect growth.

4.4 Outcomes measured

The primary outcome of all nine papers was measurement of brain structure. The secondary outcomes noted in a few of the studies were:

- Height
- Head circumference
- Social function
- Self-concept
- Speech
- Tissue composition
- Intelligence quotients

4.5 Results

4.5.1 Cerebrum and Cerebellum

The findings of nearly all the papers would suggest that the cerebrum and cerebellum were smaller in the case of NSCLP affected individuals, although the findings are somewhat equivocal. For instance, within the cerebrum, the frontal lobe was found to be decreased in the studies by Nopoulos *et al*. (2000, 2007), but increased in another paper by the same authors Nopoulos *et al*. (2002). In the case of the cerebellum, the volume was also found to be decreased in a number of studies (Nopoulos *et al*., 2002; Nopoulos *et al*., 2007; Conrad *et al*., 2010; Devolder *et al*., 2013), particularly the posterior cerebellum, but with the anterior lobes appearing larger (Nopoulos *et al*., 2000; Nopoulos *et al*., 2007; Devolder *et al*., 2013). As the cerebellum is linked with speech, abnormalities in volumes have been suggested to being linked to speech difficulties in individuals with CLP (Conrad *et al*., 2010).

In the study by Devolder *et al*. (2013), males demonstrated an overall reduction in cerebellum volume with no regional changes. While females showed no change in size when compared to the controls, in another study some regional changes were noted in the case of NSCLP affected females (Conrad *et al*., 2010). In the case of males with CPO, there were only regional changes, while females with CPO exhibited a reduced overall volume of the cerebellum (Devolder *et al*., 2013). The results would seem to suggest that some cerebellar differences might be dependent on the cleft type and gender.

4.5.2 Tissue distribution

When considering the cerebrum, tissue distribution is also different in the brains of individuals with CLP. In boys the cortical grey matter volume was reported to be larger, but the white matter volume was reduced. However, the proportions of white and grey matter were the same as the controls in girls with CLP (Nopoulos *et al*., 2007; Adamson *et al*., 2014). Not only was the white matter volume smaller, but hemisphere specific patterns of cortical volume and thickness were also noted, where total white matter volumes were shown to be lower in boys with right sided clefts compared with left sided clefts and healthy controls. Reduction in white matter volume was evident in both the cerebellum and cerebrum, but within the cerebrum specifically, it was more pronounced in the frontal and occipital lobes (Van der Plas *et al*., 2010; Adamson *et al*., 2014). By contrast in the study by Nopoulos *et al*. (2000) no significant difference in the grey and white matter ratios were reported.

4.5.3 Temporal lobe

In the case of the temporal lobe, this was reported to be smaller in two studies (Nopoulos *et al*., 2000; Nopoulos *et al*., 2002), along with a decreased size of subcortical nuclei and the occipital lobe (Nopoulos *et al*., 2000; Nopoulos *et al*., 2007).

4.5.4 Ventral frontal cortex

While the size of the VFC (this is composed of the orbitofrontal cortex and the straight gyrus) was reported to be smaller in both volume and surface area in the case of NSCLP affected individuals, principally in the left straight gyrus (Boes *et al.,* 2007), another study found no such difference in structure (Nopoulos *et al.,* 2005). Two published studies found no morphological

abnormalities in the straight gyrus, but a reduction in the orbitofrontal cortex volume and area (Nopoulos *et al.,* 2005; Boes *et al.,* 2007). Both papers linked abnormal VFC measures to social dysfunction in NSCLP patients. Boes *et al.* (2007) further highlighted that self-concept measures are not affected by the abnormalities found in the VFC.

In addition to possible structural differences in the various regions of the brain, it has been reported that the brains of children with NSCLP are less mature than age matched peers, with affected females having more immature brains than NSCLP affected males (Adamson *et al*., 2014).

4.6 The selection of controls

The studies included in this review recruited healthy individuals to act as controls in one of three ways: by placing advertisements in the local community newspaper (Nopoulos *et al.,* 2000; Boes *et al.,* 2007; Nopoulos *et al.,* 2007; Devolder *et al.,* 2013), directly from a registry in the University of Iowa Mental Health Clinical Research Center (Nopoulos *et al.,* 2000; Nopoulos *et al.,* 2002; Nopoulos *et al.,* 2005), or via local schools, where the families were contacted to discuss their willingness to participate (Adamson *et al.,* 2014).

The controls chosen were matched to NSCLP patients by age (Nopoulos *et al.,* 2002; Nopoulos *et al.,* 2005; Boes *et al.,* 2007; Nopoulos *et al.,* 2007; Van der Plas *et al.,* 2010) and gender (Nopoulos *et al.,* 2002; Nopoulos *et al.,* 2005; Boes *et al.,* 2007; Nopoulos *et al.,* 2007). In the study by Nopoulos *et al.* (2000) all the subjects and controls were right-handed, and in the later Nopoulos *et al.* (2002) study they were matched by socioeconomic status and level of education. The controls in almost all cases, particularly in the Iowa based studies, were

matched for ethnic background and so were Caucasian (Nopoulos *et al.,* 2000; Nopoulos *et al.,* 2002; Boes *et al.,* 2007; Devolder *et al.,* 2013).

4.7 Exclusion criteria

Almost all papers stated that they examined the patients before being included in the study by a trained medical geneticist to rule out congenital syndromes. To preclude subjects with syndromic clefts that had not been previously diagnosed, patients with an IQ of less than 70 were excluded (Nopoulos *et al.,* 2007; Devolder *et al.,* 2013). Any individuals with major neurologic, psychiatric illness or a history of learning disabilities and attention-deficit/ hyperactivity disorder were also excluded during the screening process (Nopoulos *et al.,* 2000; Boes *et al.,* 2007; Nopoulos *et al.,* 2007; Van der Plas *et al.,* 2010; Devolder *et al.,* 2013). Similarly, any individuals who were reported as having a history of alcohol or substance abuse were also excluded from participating in the study (Nopoulos *et al.,* 2000).

Any subjects who had orthodontic fixed appliances fitted were excluded, since the metal appliance can create image artefacts during MRI (Nopoulos *et al.,* 2007; Conrad *et al.,* 2010; Devolder *et al.,* 2013).

In addition, the study by Conrad e*t al*. (2010) aimed to assess brain structure and its effect on speech, and so any controls with speech/language difficulties and/ or significant hearing loss were excluded from the study.

4.8 Reliability

Reliability was variable across the nine studies. In two of the studies, brain volume was measured by dividing the scans into sections and then using the BRAINS image analysis tool, along with measurements made using hand tracing. Each of the hand tracers were trained on five scans and were then tested on an independent sample of 10 scans (Conrad *et al.,* 2010; Devolder *et al.,* 2013). The regional volumes were then compared, and the tracers were required to attain interclass correlation values of at least 0.90 before tracing the study sample. The final interclass correlation scores ranged between 0.90 and 0.98 (r=0.93). In the study by Nopoulos *et al*. (2005) the two raters who traced OFC and SG using 10 scans demonstrated interrater reliability scores dependent on the area of the tracing. For the cortical grey-matter volume the reliability was 0.90 and 0.92, whereas for the grey-matter volume the scores were 0.80 and 0.85.

By contrast, there were two studies that used automated measurements of the brain to ensure validity and reliability (Nopoulos *et al*., 2000; Nopoulos *et al*., 2002). This method has previously been described by Andreasen *et al.* (1996) and was reported to be efficient in cerebral lobe measurement (Andreasen *et al*., 1994).

Apart from the study by Boes *et al*. (2007), where the social function measures using "self-description questionnaires" and the "comprehensive assessment of symptoms and history" were estimated and found to demonstrate a high internal consistency, the remaining four studies did not describe any estimates of reliability.

4.9 MRI acquisition and other assessments

Of the nine studies in the review, one used a 3T Siemens TIM Trio machine based in Melbourne, Australia to obtain the MRI scans (Adamson *et al.,* 2014). The remaining eight studies used a 1.5T GE Signa magnetic resonance scanner (General electric, Milwaukee, Wisconsin), although in one the machine initially used was a 1.5 Siemens Avanto Scanner (Siemens AG, Muenchen, Germany). The reliability of both machines was tested when switching from one scanner to the other during the study and the measures were found to be comparable (Devolder *et al.,* 2013).

Processing of the acquired images was carried out using BRAINS software, where a 3-dimensional (3D) data set is formed, realigned, re-sampled and transformed into a 3D co-ordinate system known as Talairach space. Also known as Atlas, this system is used to map the brain and allow examination and measurement of its structures. This was used in all of the studies except for the one by Adamson *et al.* (2014) where the images were processed using the FreeSurfer 5.1.0 program, which segments images automatically.

Some statistical analyses were performed using SAS language with SAS STAT procedures (SAS Institute Inc, Cary, North Carolina), where brain tissue measures were analysed using multivariate analysis of variance (MANOVA) (Nopoulos *et al*., 2002; Nopoulos *et al*., 2007) or Analysis for Covariance (ANCOVA) (Nopoulos *et al*., 2000; Nopoulos *et al*., 2005). The remainder of the analyses were executed using Statistical Package for Social Sciences (SPSS)15.0, 17.0 and 19.0 for Windows, respectively (Conrad *et al.,* 2010; Van der Plas *et al.,* 2010; Devolder *et al.,* 2013). Structural measurements made in centimetres were compared using analysis of

covariance (ANCOVA) to determine whether there are any significant differences between two independent groups on a dependent variable. In the study by Boes *et al.* (2007), the analyses were performed using SPSS 13.0 for Windows and the covariates were accounted for using MANCOVA. In the Australian study, a mass univariate one-way analysis was conducted on collections that exhibited a significant effect (Adamson *et al.,* 2014). Other measures included neuropsychological tests to measure IQ (Nopoulos *et al*., 2002; Nopoulos *et al*., 2005; Nopoulos *et al*., 2007; Devolder *et al.,* 2013), measurements of height and head circumference (Nopoulos *et al*., 2007; Devolder *et al.,* 2013), assessment of social function using questionnaires, and speech assessment by experienced speech pathologists (Boes *et al*., 2007; Conrad *et al.,* 2010).

4.10 Summary tables

Tables 3 and 4 provide a summary of the information from each of the studies identified for this systematic review. Table 3 provides a summary of the characteristics of papers included in qualitative and quantitative synthesis, including sample size, cleft phenotype and outcome measures.

Study ID	Author, Date, Study Design	Cleft Group	Control Group	Type of Cleft	Geographical location	Outcome measures
1	Nopoulos *et al.,* 2007 Case Control	74	74	CLO (n=18), CLP (n=33), and CPO (n=23).	Iowa	Brain structure
2	Van der Plas *et al.,* 2010 Case Control	33	57	14 right CLP and 19 left CLP	Iowa	Brain structure
3	Nopoulos *et al.,* 2002 Case Control	46	46	32 subjects with CLP (11 with bilateral clefting, 18 with left unilateral clefting, 3 with right unilateral clefting) and 14 subjects with CPO	Iowa	Brain structure
4	Devolder *et al.,* 2013 Case Control	107	127	31 CPO (n=31), CLP (n=54), CLO (n=22)	Iowa	Brain structure
5	Adamson *et al.,* 2014 Case Control	26	26		Melbourne	Brain structure
6	Nopoulos *et al.,* 2000 Case Control	14	14	CPO (n=5), CLP (n=8), CLO (n=1)	Iowa	Brain structure
7	Conrad *et al.,* 2010 Case Control	43	43	NSCL (n=7), NSCP (n=11), and NSCLP (n=25)	Iowa	Brain structure alteration and relationship to speech
8	Boes *et al.,* 2007 Case Control	30	43	CLO (n=8), CLP (n=15) and CPO (n=7)	Iowa	Social function and brain structure
9	Nopoulos *et al.,* 2005 Case Control	46	46	32 subjects with CLP (11 BCLP, 18 with left UCLP, 3 with right UCLP) and 14 CPO	Iowa	Social function and brain structure

Table 3: Summary characteristics of papers included in qualitative and quantitative syntheses.

Study ID	Meta-analysis ID	Author, Date	Outcome measured	Mean (SD) For Cases vs Controls in volume (cm^3)	M/F	Comments
1	**1**	Nopoulos *et al.,* 2007	Total cerebellar volume	128.0 (SD 13.0) vs 138.0 (SD 13.1)		
2		Van der Plas *et al.,* 2010	Total cerebellar volume	133.20 vs 142.80	M	Adjusted mean to balance data for cleft on right side
2		Van der Plas *et al.,* 2010	Total cerebellar volume	138.0 vs 142.80	M	Adjusted mean to balance data for cleft on left side
3	2	Nopoulos *et al.,* 2002	Total cerebellar volume	133.0 (SD 17.7) vs 146.0 (SD 16.9)	M	
4	3	Devolder *et al.,* 2013	Total cerebellar volume	141.3 (SD 14.1) VS 151.3 (SD 12.8)	M	Control and test for males
4	4	Devolder *et al.,* 2013	Total cerebellar volume	131.7 (SD 14.3) vs 138.0 (SD 10.7)	F	Control and test for females
5		Adamson *et al.,* 2014	Total cerebellar volume			Only mean volume present after correction
6	5	Nopoulos *et al.,* 2000	Total cerebellar volume	123.6 (SD 13.8) vs 135.5 (SD 15.5)	M	
7	6	Conrad *et al.,* 2010	Total cerebellar volume	127.5 (SD 13.06) vs 143.25 (SD 10.23)	M	Control and test for males
7	7	Conrad *et al.,* 2010	Total cerebellar volume	118.95 (SD 8.92) vs 128.69 (SD 9.62)	F	Control and test for females
8	8	Boes *et al.,* 2007	Frontal cortex and straight gyrus	78294.0 vs 80568.0	M	Control and test for frontal cortex
8	9	Boes *et al.,* 2007	Frontal cortex and straight gyrus	1.87 vs 2.10	M	Control and test for straight gyrus

Table 4: Outcome measures and their results as mean and standard deviation. The study ID is the same as used in Table 3.

9	10	Nopoulos *et al.,* 2005	Frontal cortex and straight gyrus	36.0 (SD 5.04) vs 36.9 (SD 6.19)	M	Control and test for frontal cortex volume
9	11	Nopoulos *et al.,* 2005	Frontal cortex and straight gyrus	5.26 (SD 0.88) vs 4.85 (SD 1.01)	M	Control and test for straight gyrus volume
3	12	Nopoulos *et al.,* 2002	Cortical grey matter	652.0 (SD 67.2) vs 649.0 (SD 59.7)	M	
5		Adamson *et al.,* 2014	Cortical grey matter			Only mean volume present after correction
1	13	Nopoulos *et al.,* 2007	Intracranial volume	1378.0 (SD 125.0) vs 1449.0 (SD 109.0)		
2		Van der Plas *et al.,* 2010	Intracranial volume	1368.90 vs 1447.40	M	Adjusted mean to balance data for cleft on right
2		Van der Plas *et al.,* 2010	Intracranial volume	1402.80 vs 1447.40	M	Adjusted mean to balance data for cleft on left
3	14	Nopoulos *et al.,* 2002	Intracranial volume	1449.0 (SD 141.0) vs 1490.0 (SD 117.0)	M	
6	15	Nopoulos *et al.,* 2000	Intracranial volume	1426.5 (SD 111.3) vs 1417.2 (SD 116.0)		
1	16	Nopoulos *et al.,* 2007	Total cerebral volume	1198.0 (SD 112.0) vs 1253.0 (SD 96.0)		
2		Van der Plas *et al.,* 2010	Total cerebral volume	1198.10 vs 1198.70	M	Adjusted mean to balance data for cleft on right
2		Van der Plas *et al.,* 2010	Total cerebral volume	1200.80 vs 1198.70	M	Adjusted mean to balance data for cleft on left
3	17	Nopoulos *et al.,* 2002	Total cerebral volume	1190.0 (SD 123.0) vs 1201.0 (SD 108.0)	M	
6	18	Nopoulos *et al.,* 2000	Total cerebral volume	1234.6 (SD 98.2) vs 1209.4 (SD 105.5)	M	

Table 4 (cont.) Outcome measures and their results as mean and standard deviation (note all Meta-analysis studies were conducted at the same institution - University of Iowa)

4.11 Results of the meta-analysis

Of the nine studies identified in this review, seven were included in the meta-analysis (Table 2). Two articles, study 2 (Van der Plas *et al.,* 2010) and study 5 (Adamson *et al.,* 2014), were excluded due to an absence of raw data. The authors were contacted but were unable to provide the data. In order to be included in the data analysis, the minimum data requirements were sample size, mean and standard deviation, or sufficient statistical data to allow these to be calculated. The data used in the analysis is in Table 4, together with the different outcome measures reported in each of the seven studies.

Figure 8 is a forest plot of brain structure for the 18 outcomes, as one single plot, comparing NSCLP affected individuals and controls for the seven studies included in the meta-analysis. The summary diamond within the forest plot does not cross the line of no effect (shown in green), indicating that the presence of a cleft has a statistically significant effect on brain structure in NSCLP affected individuals, when compared to controls. This is shown by Hedge's g where it is used to calculate effect size and 95% confidence intervals (-0.42 [CI -0.61, -0.22]), which do not include zero and confirms a statistically significant effect. Theta is the 'effect size' where a statistical model measures the strength of the relationship between two variables and for this data if $\theta = 0$ then there is no statistically significant effect. The lower and upper confidence intervals (CI) about θ do not include 0 so we can again conclude that there is an effect. The formal statistical test has $p = 0.0001$ so we can reject the null hypothesis of no effect at $\alpha = 0.05$. A prerequisite for the use of the random-effects model used in this analysis is homogenous data. τ^2 is a measure of heterogeneity and if $\tau^2 < 0.25$ then there is 'small'

heterogeneity. In this case τ^2 =0.119. However, the somewhat subjective value of I^2 = 70.98% ($p<0.001$) would suggest there might be substantial heterogeneity (Higgins *et al.,* 2019). I^2 is a statistic which denotes the percentage of heterogeneity in a meta-analysis. This heterogeneity can be as a result of differences in the study populations, such as the different outcomes, or methodology used, resulting in confounding bias, selection bias and even perhaps publication bias. Therefore, to further explore the potential effect of clefting on brain structure, each of the five outcomes (Table 4) were investigated separately and the results are shown in Figure 9.

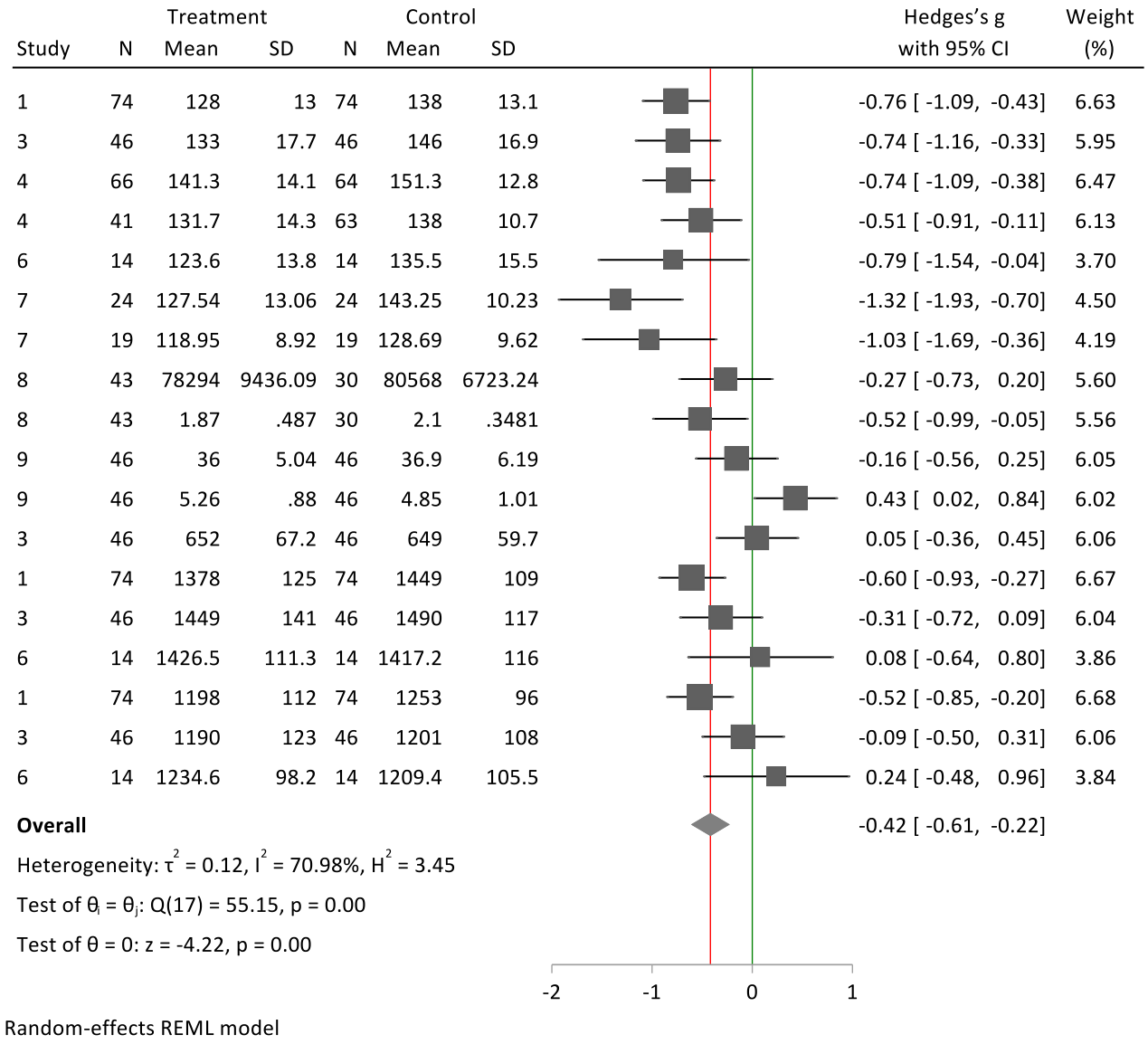

Figure 8. Forest plot of the data for each of the outcomes in the seven studies listed in Table 4 for Treatment (NSCLP) and Control (non-CLP).

Figure 9 is a composite of the summary plots for the subgroup analyses for the five outcomes, namely: Cortical grey matter, Front cortex and straight gyrus, Intracranial volume, Total cerebellar volume and Total cerebral volume. These will be described in turn.

- **Cortical grey matter.** There is only one study and so the forest plot is of little use. However, it is worthwhile noting that this study showed that the cortical grey matter was increased in NSCLP patients (Nopoulos *et al.*, 2002).

- **Frontal cortex and straight gyrus.** The summary diamond crosses the line of no effect, with the value of Hedges's g and the 95% confidence intervals (-0.12 (CI -0.52, 0.28)) including zero, confirming there is no statistically significant effect of clefting on the frontal cortex and straight gyrus. However, the formal statistical test for Theta has a p = 0.02, suggestive of an effect. Once again, a prerequisite for the use of the random-effects model used in this analysis is homogenous data. In this case τ^2 =0.12, but the value of I^2 = 69.99% would suggest substantial heterogeneity. It is worth noting only two studies are included here, namely study 8 (Boes *et al.,* 2007) and study 9 (Nopoulos *et al.,* 2005).

- **Intracranial volume.** The summary diamond is to the left of the line of no effect and does not cross it. Overall, the value of Hedges's g and the 95% confidence intervals (-0.38 (CI -0.71, -0.06)) do not include zero, indicating there is a statistically significant effect of clefting on Intracranial volume. However, some caution should be exercised here because although τ^2 =0.03 and the formal statistical test has a p=0.19, which suggest we can assume the dataset is homogenous, the I^2 = 36.78% would still suggest moderate heterogeneity.

- **Total Cerebellar volume.** Once again, the summary diamond is to the left of the line of no effect, and this was the case for the means and 95% confidence intervals of all the studies reporting on this feature. The overall value of Hedges's g and the 95% confidence intervals (-0.77 (CI -0.94, -0.60)) did not include zero. Therefore, there would seem to be a statistically significant effect of clefting on Total Cerebellar volume, with it being smaller in the case of cleft affected individuals compared to unaffected controls. This dataset was also found to be homogenous with τ^2 =0.001, p=0.51 and I^2 = 0.001%.

- **Total Cerebral volume.** The summary diamond crosses the line of no effect and so there is no statistically significant effect of clefting on Total Cerebral volume, which is confirmed by the value of Hedges's g and the 95% confidence intervals (-0.21 (CI -0.62, 0.21)) including the value zero. Although τ^2 =0.08, the value of I^2 = 56.67 % would suggest substantial heterogeneity.

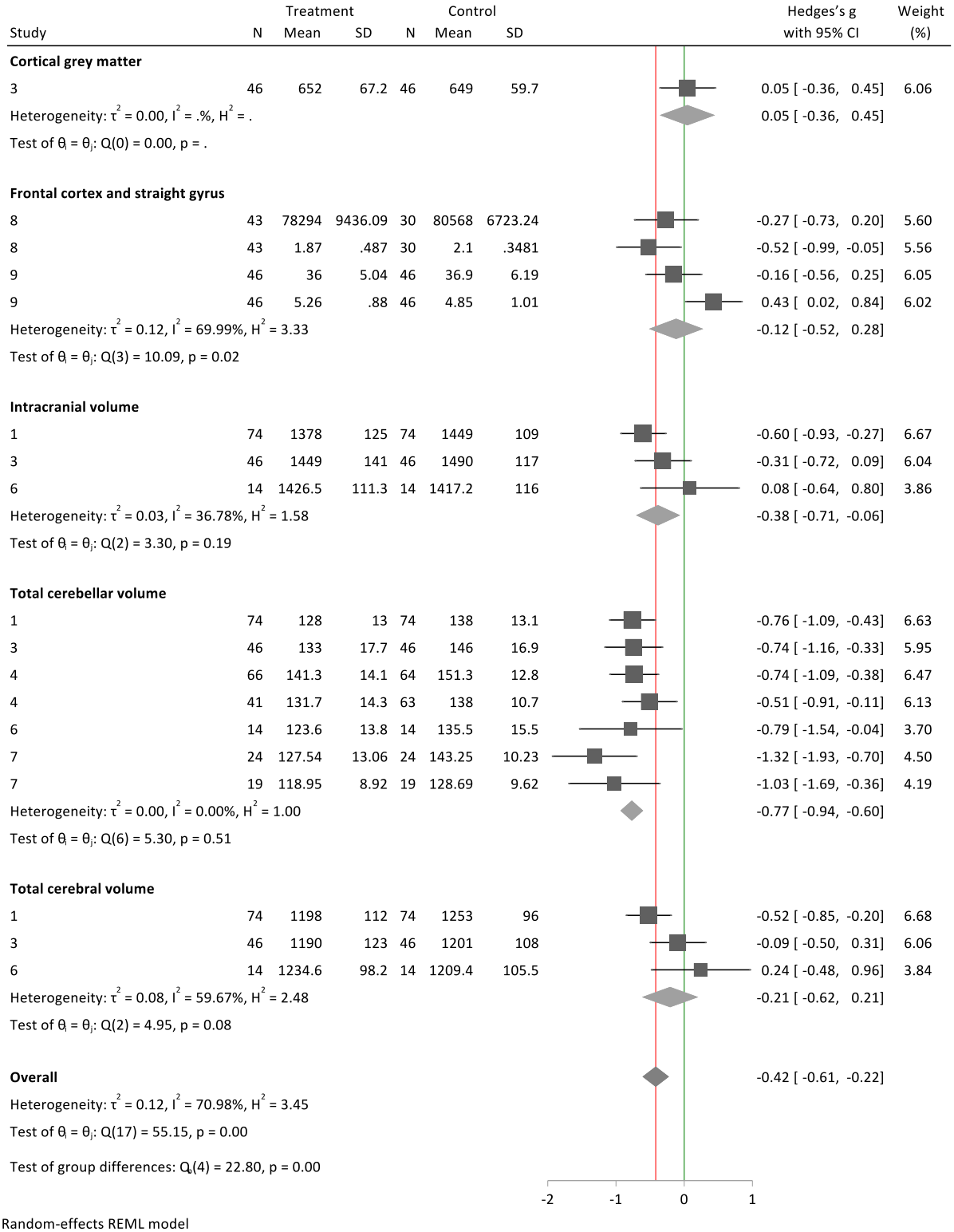

Figure 9. Forest plot of the data for each of the outcomes (NSCLP and non-CLP) in the seven studies listed sub-grouped in Table 4 (Cortical grey matter, Frontal cortex and straight gyrus, Intracranial volume, Total cerebellar volume and Total cerebral volume)

4.12 Risk of Publication Bias

Publication bias occurs when only positive results are published and proves that a study has confirmed that the hypothesis is true. As a result, this can mean that studies included in a meta-analysis are biased due to including more trials with positive findings over those with negative findings. Therefore, the results appear to be skewed and such results will not be a true representation of the actual occurrence of a disease in a population**.** Funnel plots are often used to identify the presence of such bias. Figure 10 shows that the effect sizes are not symmetrically spread across the plot, with some lying outside the 95% boundary, suggesting that some publication bias possibly exists.

This can also be due to chance or poor study quality. In addition, to help identify which specific structures participate in the asymmetry, more visual contour enhanced funnel plots were used to detect publication bias due to the suppression of non-significant findings. The dark grey region corresponds to p-values greater than 0.10 and the white region (outside the funnel) corresponds to p-values below 0.01. Figure 11 illustrates funnel plots for the sub-grouped measures. The cortical grey matter and frontal cortex and straight gyrus shows a symmetrical funnel plot, while intracranial volume, total cerebellar volume and total cerebral volume show asymmetrical funnel plots.

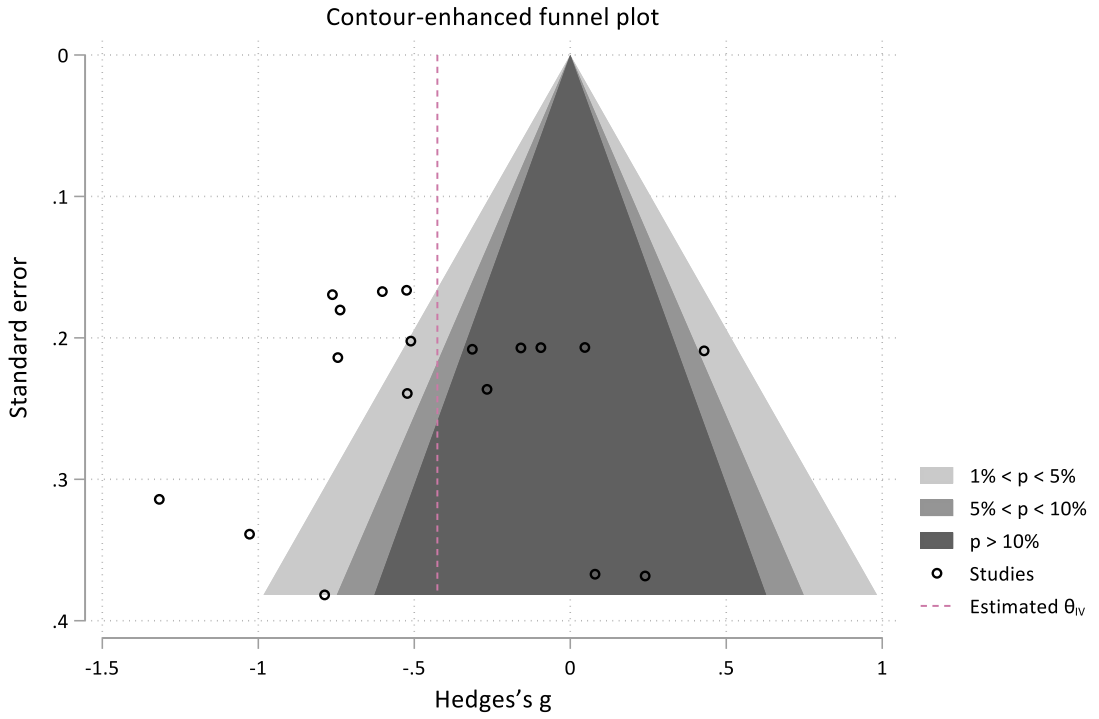

Figure 10. Contour-enhanced funnel plot for the assessment of publication bias (all studies).

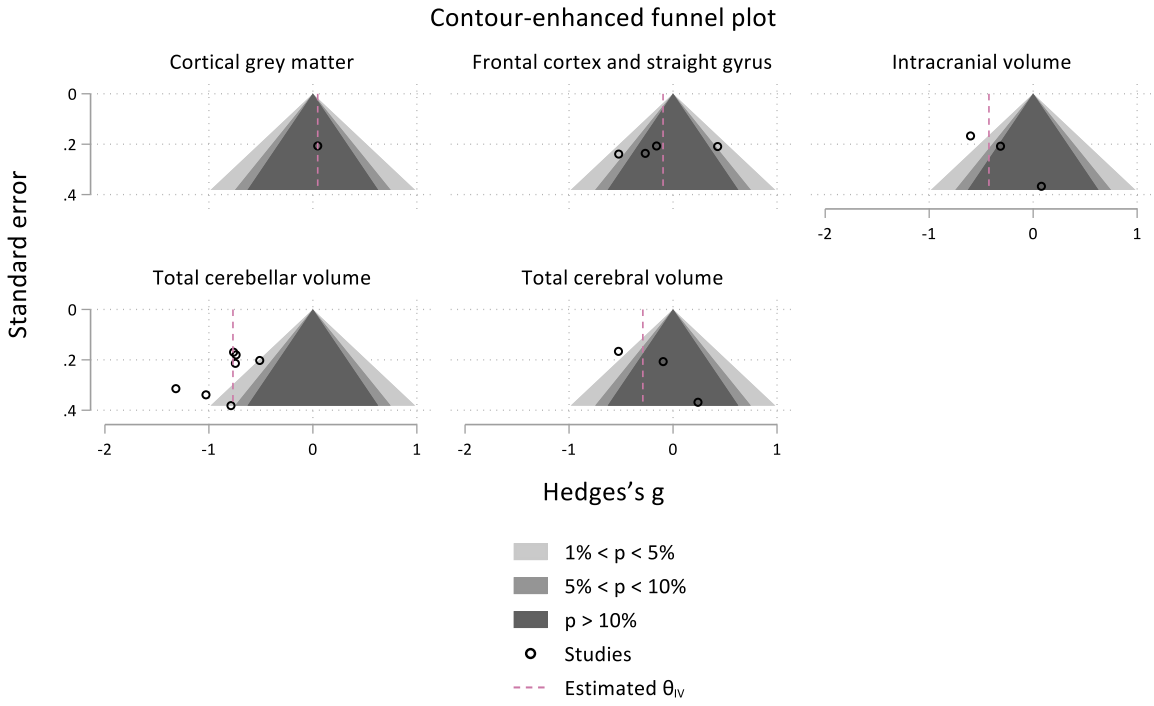

Figure 11. Contour-enhanced separated funnel plots for the assessment of publication bias for each of the subgroup.

5. Discussion

The aim of this meta-analysis was to determine whether the presence of a cleft influences brain morphology of affected individuals. To date there has been no previously published meta-analysis on the potential relationship between cleft lip and/or palate affected individuals and altered brain structure.

The first part of the discussion will cover the key outcomes of this review and after this the strengths and limitations will be described highlighting any methodological issues. Finally, the implications of the findings on the management of cleft affected individuals will be discussed.

5.1 Summary

Nine studies fulfilled the inclusion criteria for this systematic review, and seven were used for the meta-analysis. As each paper reported on a variety of structures, several structures were combined under specific subgroups for the meta-analysis for ease of reporting. These subgroups were: cortical grey matter, frontal cortex and straight gyrus, intracranial volume, total cerebellar volume and total cerebral volume.

For the three subgroups, cortical grey matter, frontal cortex/ straight gyrus, and total cerebral volume, the summary diamond on the forest plot crossed the line of no effect, indicating no statistically significant effect of clefting on the sizes of these structures. By contrast there was a statistically significant effect of the presence of a cleft in the case of intracranial volume and total cerebellar volume. However, the results should perhaps be viewed with a degree of caution as will now be discussed.

5.2 Publication bias

The aim of the current review and meta-analysis was to summarise the evidence presented on the relationship if any on the presence of a cleft and altered brain structure. This was carried out only on published data, and consequently there is chance there is publication bias in this review, as unpublished studies might be present and would not have been included. The findings may therefore have over or underestimated the effect of the presence of a cleft on altered brain structure. The funnel plots in the current study show that there is the likelihood publication bias is present. Therefore, some degree of caution is required when interpreting the results.

5.3 Strengths and limitations of this review and meta-analysis

To help minimise bias in the selection of the papers for this review, and subsequent meta-analysis, a strict protocol was used which included a specific research question, search methodology, data collection and data extraction. The search was completed using two database search engines as well as an internet search engine (Google scholar), and hand searching of the reference lists of full text articles. The reviewers determined separately which articles should be included, and any conflict was resolved by a third reviewer to decide if a study should be included. This will have helped to reduce selection bias (Boutron *et al*., 2019). However, it is difficult to eliminate bias entirely and in this review the search only included published studies, and those published in English.

When looking more specifically at the potential strengths of the papers included, in a number of respects there were common strengths in the reviewed papers. These included focused aims, clear descriptions of the cleft lip and palate subjects, clearly labelled interventions and outcome

measures. Consequently, when using the CASP tool for systematic reviews (appendix B) it was easy to note whether the included papers were suitable to be included in the review. The CASP tool is an efficient and straight forward checklist used to assess the validity of the results reported in a paper. Also, what the results are and whether or not any observed effect applies to the local population. From the information assessed through the tool, the papers were assessed for rationality of results, if the controls were selected in an acceptable way, how large the treatment effect was and if the results match the current existing evidence.

A challenge when comparing the results of the studies was that the demographics of the cleft groups were sometimes different. For example, in some studies the cleft subjects were all male and in others it was a mixture of males and females, which is a potential confounder. Another potential confounding factor was the age groups chosen in certain studies. Some included only subjects above 18 years of age, whereas other papers included children.

A further potential limitation of the meta-analysis is that the papers included in this part of the research were all from the University of Iowa, such that we do not know if the results are generalisable. Although the systematic review itself contained one Australian study, it wasn't included in the meta-analysis due to missing raw data and insufficient summary data. Also, the meta-analysis was done on studies that included mainly Caucasians participants and the results may have been different if other ethnic groups had been included.

5.4 Strengths and limitations of the imaging techniques used

When considering the technologies used in the studies, principally MRI, there were no reports of any risks encountered during this intervention and none stated there were any legal or ethical issues. MRI has been in use for many years and all publications made it clear that patients and controls signed a consent before being involved in any study. However, it was unclear from all but one of the studies, whether the person evaluating or analysing the outcomes was blinded as to the grouping. In only one study were the technologists performing the image analysis described as being "blinded" as to the grouping of the participants (Nopoulos *et al.,* 2007). There was also a lack of evidence in the methodological reliability of analysis of the results, which in turn leads to questions as to the validity of the results observed.

An interesting limitation was that participants were excluded if they had an IQ below 70 (Nopoulos *et al*., 2007; Devolder *et al*., 2013). This may have excluded more NSCLP affected individuals than controls, and therefore any reduction in the IQ would be have been related to marked structural abnormalities in the cleft cases. This would have introduced an element of selection bias.

5.5 Selection and recruitment of the control group

Even though CLP is conventionally thought of as an anomaly that causes structural abnormalities limited to the soft and bony tissues of the face and oral cavity, there is some recent evidence to suggest there maybe cognitive and neuroanatomical effects that extend to altered brain morphology (Nopoulos *et al.,* 2002a, 2007; Shriver *et al.*, 2006). To determine this requires careful selection of any comparative to minimise recruitment bias. This is because

there are known confounders such as socioeconomic status, age, dietary intake, gender and other genetic associated syndromes that affect the brain. Wherever possible the studies that were included tried to match the cleft and control groups in terms of age, gender and socioeconomic status, as well as ethnic grouping.

Most studies reported with matched case and control groups, but two studies had fewer participants in the cleft group than the control group (Boes *et al*., 2007; Van der Plas *et al.,* 2010). This might have been due to difficulties in identifying sufficient cases for inclusion in the cleft group. Nevertheless, the ratio was still less than 1:2 which is not unreasonable, and the numbers were sufficient for a statistical analysis to be able to detect any differences (Linden and Samuels, 2013). It was also unclear if any randomisation had been applied in the selection of the participants to the studies, although again it may have been that decisive sampling of the cleft cases was required because of the limited numbers available.

Most of the control group were recruited via newspaper advertisements, which presumes not only that the cleft affected individuals or their parents buy newspapers, but that they were able to read and interpret the information about the study. There was therefore the potential of recruitment bias in terms of literacy and finance in being able to purchase the newspapers. If so, the control group may have had higher IQs and might have been from different socio-economic groups.

5.6 Reporting of methodology

The methodological processes used in each of the studies included in the review were similar, largely because most of them were carried out in the same hospital at the University of Iowa in the USA, and by the same workers. Nonetheless, there were small differences in the methodologies particularly with respect to the cases and controls. For instance: Nopoulos *et al.* (2007) evaluated brain structure in NSCLP patients and considered both males and females; Nopoulos *et al.* (2000) and Nopoulos *et al.* (2002) evaluated brain morphology in adult NSCLP males; Devolder *et al.* (2012) looked at dissimilar CL/P phenotypes and the potential effect on the cerebellar structure in both males and females; Conrad *et al.* (2010) investigated whether or not speech is affected by altered structural differences in the cerebellar structure in NSCLP male and female patients; and Nopoulos *et al.* (2005) and Boes *et al.* (2007) considered social function in boys with NSCLP and the possible relationship with ventral frontal cortex morphology.

All publications reported that the NSCLP patients included in the studies had been previously examined by a trained geneticist in order to rule out congenital syndromes. However, little detail was provided as to the examiner(s) who made the judgement and if a further examiner was available to assess if the decision on selecting patient's enrolment was biased. This would have further increased the potential for recruitment bias which might have affected the outcome of this review.

Of the seven studies included in this meta-analysis, five described estimates of reliability; three studies reported calibration of examiners, two studies carried out automated measurements

and the other two failed to report reliability testing or calibration. However, it should be remembered that the lack of reporting does not mean that these steps were not taken. This highlights the importance of following a specific checklist for publications, such as having an order of introduction, clearly stated materials and methods, results and discussions stated in a logical order and giving a sensible straight forward conclusion (Mack, 2015).

With respect to image analysis, Nopoulos *et al.* (2002; 2005; 2007) employed subcortical structure volumes obtained using an automated neural net, where pure grey matter was represented (Magnotta *et al.,* 1999) and tissue volumes were broken down into sections (Harris *et al.,* 1999). However, Devolder *et al.* (2012) stated that even though the neural network automatically detected the cerebrum and cerebellum and subdivided it, the measures were not scrutinised because of the relatively low resolution of the images (1.5 T GE) and complex branching of the cerebellar white matter structure in this study.

5.7 Geographical location

As formerly indicated, the seven studies included in the meta-analysis were carried out in Iowa and the main ethnic group was Caucasian.

The reason these studies were on Caucasians are perhaps twofold. Firstly, Caucasians most likely represented the largest proportion of the general population at that time in Iowa and secondly, the incidence of CL/P in populations of Africans origin is low (Lidral *et al.,* 2008). Although the incidence of CLP in native Americans is relatively high at 3.6 in 1000 births this

group were not represented in their studies, perhaps because the total population of native Americans in the state is small.

Research has revealed that low SES has an increased likelihood of impacting the health status of a family, and this applies to both developing and developed countries (Jednoróg *et al.,* 2012). Although Iowa is a developed state in a developed country (United States), individual SES levels tend to affect subjects separately, and so influence the brain structure and maturaty level. This requires further investigation as it might have had an effect on altered brain structure found in CLP patients. Links between low SES and altered brain structure have previously been reported, with individuals within the lower SES groups sometimes presenting with structural dissimilarities within the brain (Brito and Noble, 2014).

As all of the studies incorprated in this meta-analysis were based in Iowa and the stated aim in all the included papers was to match the SES of the parents cases and controls, the risk of the SES affecting the reliability of the interpretation of the outcomes of this systematic review and meta-analysis are low.

5.8 Participant's age

The studies included in this review and data analysis considered both child and adult participants. The growth and development of the human brain is lengthy, and even though 95% of the volume of the cerebrum has formed by the age of 5 years, changes still take place within the white and grey matter until puberty and early adulthood (Sowell *et al.,* 2003). Therefore it is reasonable to expect that structural differences might be found in the brains of developing children, particularly when they can develop at different rates, and when compared to adults,

irrespective of the presence of a cleft. This is because the brain is still growing and will not have acquired its full size in the child. Nevertheless, the cerebellum remained abnormally small, and until a later age, in cleft affected individuals when compared with age matched controls. The studies identified in this review were all cross sectional, and ideally studies need to assess subjects longitudinally in order to further understand how the NSCLP patient's brain matures when compared to unaffected, healthy brains. It would certainly be useful to map structural development of the brain throughout the childhood and early adulthood using MRI techniques in both case and control subjects.

In most of the reports, age was considered to be a covariate in order to minimise the effect of age on the brain size and morphology between different age groups (Giedd *et al*., 1996). The breakdown of the ages and genders of the participants of publications included in the meta-analysis are shown in Table 5.

5.9 Participant's Gender

Gender might have an effect on brain structure in those born with a cleft (Nopoulos *et al.,* 2007). It is common to see gender differences in conditions that specifically affect the brain such as autism, mental retardation, attention deficit disorder and dyslexia (Nopoulus *et al.,* 1997; Broman *et al.,* 2013), all of which are more common in males. We already know that the incidence of CL/P is greater in males (2:1 relative to females), albeit that CPO is more common in women. This systematic review and meta-analysis has shown that brain structure, notably intracranial volume and total cerebellar volume are different in cleft affected individuals compared to the controls. Even though both NSCLP females and males demonstrated a reduced

cerebral volume, males with NSCLP demonstrated an additional abnormality compared to unaffected controls, namely a greater cortical volume and reduced cerebral white matter (Nopoulos *et al.,* 2007). As most of the subjects in the studies were males then an association between CL/P and brain structure is potentially biased to that gender.

Four papers in this review included males only, and the remaining three case control studies comprised both females and males, where the larger percentage of participants were males (Table 5). A possible reason is that twice as many males as females are affected with CL/P and this may account for the lower number of females included.

With respect to cognition it is known that there is asymmetry of the brain. The male brain processes language function unilaterally (left sided), while the female brain has a more bilateral representation (Shaywitz *et al.,* 1995). This might be due to differences found in early brain development, where its hypothesised that the testosterone either promotes the right hemisphere or slows down maturity in the left hemisphere (Bear *et al*., 1986). This might possibly explain the male 'vulnerability' in terms of neurodevelopmental syndromes, namely language disorders (dyslexia) (Geschwind *et al.,* 1985). It is not known if the same could be true for cleft development.

Study included	Gender of participants	Age of participants
Nopoulos *et al.,* 2007	Males: 100 Females: 48	7 to 17 years old
Nopoulos *et al.,* 2002	92 Males (no females)	Above 18 years old
Devolder *et al.,* 2013	Males: 130 Females: 104	7 to 27 years old
Nopoulos *et al.,* 2000	28 Males (no females)	Above 18 years old
Conrad *et al.,* 2010	Males: 72 Females: 57	7 to 17 years old
Boes *et al.,* 2007	73 Males (no females)	7 to 12 years old
Nopoulos *et al.,* 2005	92 Males (no females)	Above 18 years old

Table 5: Summary of demographics in papers used for the meta-analysis.

Not all of the studies included in this review demonstrated what specific structural abnormality patterns are associated with gender in clefts. Even though the reported differences might be small compared to the overall brain structure, the studies included in this systematic review do suggest some differences with age and gender on brain structure, and it is possible that this might impact on cognition. However, further research is necessary to determine if such structural differences do result in cognitive and/or behavioural abnormalities.

5.10 Cleft phenotype

With respect to cleft phenotype and brain tissue volume, the greatest volume was seen in the controls, followed by the CLO group, CPO group and the smallest was seen in the CLP group (Nopoulos *et al*., 2007). These results are similar to those reported by Weinberg *et al.* (2009) where variations were found between CPO and CLP affected groups when compared to controls. A study by Chollet *et al.* (2014) showed that CPO was associated with cerebral heightening, narrowing of the frontal lobe and reorientation of the Broca's area, whereas the presence of CLP was linked with shifts in the occipital lobes, temporal lobes and shortening of the cerebellum. Likewise, Nopoulos *et al.* (2007) observed a reduced volume in the case of the frontal lobe in CL/P patients relative to controls. In the early stages of embryonic development, during the formation of the primary palate, failure of posterior and superior positioning might possibly cause a certain discrepancy in the neural tissue distribution, conceivably only limited to CLP patients (Nopoulos *et al.,* 2000). By contrast, the secondary palate develops during the late embryonic stage when the vertical separation between the face and the brain has already occurred and so any effects on neural development might be different in CPO affected individuals compared with CLP affected individuals. It is worthwhile noting however that this might not be a universal finding. Nopoulos *et al.* (2002) did not find any differences in patterns of structure between adults with CLP and CP.

Anxiety, depression and decreased social functioning might all play a role in the cognition and speech problems which are seen in NSCLP patients (Hunt *et al.,* 2005). Early studies have shown that CPO patients not only have difficulties with language but also exhibit greater reading

disabilities (Richman, 1980; Richman *et al.,* 1988). Language is certainly a complex function facilitated by a variety of regions of the brain. Although the purpose of the shape analyses of the brain conducted in the studies within the review were to look for structural and not functional effects, it would be reasonable to assume a relationship between structural effects related to cleft and its phenotypes and cleft-specific language insufficiencies if the structural effects are seen within the primary language area of the brain.

Genetic factors possibly guide specific subtype anomalies and there is strong evidence that CLP and CPO have different genetic aetiologies (Grosen *et al*., 2010; Luwig *et al.,* 2012). A classic example of this is the gene Interferon Regulator Factor 6 protein, where any single nucleotide polymorphism within this gene has been found to be associated with an increased incidence of CL/P, but not CPO (Huang et al., 2009). The genetic factors that determine the different facial abnormalities within the cleft subtypes may also drive the different cerebellar irregularities seen in cleft. This perhaps further supports the claim that CL/P and CPO are distinct conditions.

In this systematic review and meta-analysis all seven papers included different cleft phenotypes (at least CLP/CPO) in their studies. However, the CPO sample was small compared to the CLP sample making comparisons between two somewhat limited.

5.11 Laterality

Cleft laterality is assessed on gross and regional abnormalities. Van der plas *et al.* (2010) reported abnormalities in the volume and white/grey matter distribution in boys with right CLP, but not in boys with left sided CLP. A more recent paper published by Gallagher *et al.* (2017) found the opposite to be true with respect to academic performance, with left sided CLP subjects achieving poorer academic performance than their unaffected classmates or right sided CLP subjects. However, it is worth noting that although the right sided CLP individual's performance scores were not statistically significantly different from the unaffected controls, their performance with respect to language and mathematics was still slightly lower (Wehby *et al.,* 2014).

5.12 Summary of findings

This systematic review and meta-analysis is the first of its kind to summarise the findings of previous studies investigating the potential relationship between brain structure and the presence of NSCLP. The principal findings were:

- There was a statistically significant effect of cleft on intracranial volume
- There was a statistically significant effect of cleft on total cerebellar volume

This has highlighted that NSCLP affected individuals demonstrate areas of brain dysmorphia when compared to unaffected controls. The pattern of dysmorphology varies by:

- Cleft phenotype
- Gender
- Age

The structural abnormalities identified in the meta-analysis principally from papers of Nopoulos and co-workers suggest that NSCLP is a neurodevelopmental disorder (Nopoulos *et al*., 2005,2007; Weinberg *et al*., 2009; Van der plas *et al.*, 2010). The abnormal tissue distribution among neural lobes and the pattern of cognitive shortfalls in this population closely resembles developmental dyslexia. However, the results do not provide enough evidence that brain dysmorphology exhibited in this population is due to a deficit of neuronal migration. It is known that brain development is shaped not only by biology but also environmental exposure, including experience. Therefore it is possible that any structural or cognitive abnormalities associated with CLP, including any gender differences, are because of environment with experience-dependent changes in the brain. Considering that facial dysmorphology likely results from abnormalities in cellular migration and apoptosis, then brain dysmorphology may arise from deficits in these same processes. Evolving biological research on the molecular developments underlying brain growth in CLP and studies of brain structure in infants with CLP would shed light on this matter.

5.12.1 Limitations

Some of the limitations of this systematic review and meta-analysis have already been described and include: most of the studies included in the meta-analysis were carried out in one centre in one country, and the principal ethnic group was Caucasian, which was likely a reflection of the local population. The results are therefore perhaps not generalisable. This was perhaps compounded by only including studies published in English.

Even though CLP is a common birth anomaly, collecting a sufficient sample size to study any related neurological effects is difficult. The studies in this review had what initially appeared to be good sample sizes, but once factors such as phenotype, age and gender were taken in account the numbers were not high. Another limitation of studying the effect of cleft on brain development is that relatively few centres will have access to the facilities and funding to be able to investigate them using non-invasive MRI. The studies included in the review were all cross-sectional, cohort studies. Ideally at least some longitudinal component should have been included to assess the effect of age and growth on brain development in the cleft and control groups.

Although the research identified in this review demonstrated structural differences in the brains of cleft affected individuals, it was not able to elicit the exact cause of the dysmorphology or relate this to any cognitive effects.

5.12.2 Future research

This systematic review and meta-analysis have highlighted some deficiencies in the studies so far on the relationship between the cleft and brain structure and therefore possible avenues for future research:

1. Longitudinal as well as cross sectional studies of brain structure using MRI in cleft affected individuals. To make this feasible such studies need to be multicentre, and in reality, probably International. This would improve not only our understanding of the relationship between cleft and brain structure at single time point but also over time. It

would also provide sufficiently large sample sizes for each of the different cleft phenotypes and be able to account for gender as well as age.

2. As well as identifying structural differences in the brains of cleft affected individuals, it would be useful to know how these might be related to any cognitive deficits. This might then also help develop effective educational support interventions primarily during the formative years.
3. Research has highlighted the possibility of the brain and face being linked during development both structurally and through molecular signalling mechanisms. Despite this there is limited information on these mechanisms. Future work on the molecular processes that impact both the development of the face and the brain might inform the aetiology of orofacial clefting.

6. Conclusions

The following conclusions with respect to the potential link between the presence of CLP and altered brain structure can be drawn from this systematic review and meta-analysis:

- the presence of CL/P affects intracranial volume in that there is a statistically significant reduction compared to controls.
- the presence of CL/P affects total cerebellar volume where there is a statistically significant reduction compared to controls.
- there was no statistical significance effect for the presence of CL/P on total cerebral volume, the frontal cortex and straight gyrus, or cortical grey matter.

The quality of the papers included in this systematic review and meta-analysis are not particularly high, due to the incomplete description of examiner training and reliability testing. There were also a number of confounding factors, in particular age and gender. However, overall the presence of a cleft in NSCLP affected individuals does appear to have an effect brain structure as outlined above.

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Appendices

Appendix A: Data extraction form
Appendix B: Critical appraisal risks programme tool

Appendix A: Data extraction form

General information

1. **Date form completed *(dd/mm/yyyy)***	
2. **Name/ID of person extracting data**	
3. **Report title** *(title of paper/ abstract/ report that data are extracted from)*	
4. **Study funding source (including role of funders)**	
5. **Report author contact details**	
6. **Publication type (e.g. full report, abstract, letter)**	

Study characteristics

7. **Type of study**	
8. **Participants**	
9. **Types of intervention**	
10. **Types of outcome measures**	
11. **Reason for exclusion**	

Population and Settings

12. **Population description** *(from which study participants are drawn)*	

13. **Setting** *(including location and social context)*	
14. **Inclusion criteria**	
15. **Exclusion criteria**	
16. **Method/s of recruitment of participants**	

Methods

17. **Aim of study**	
18. **Design** *(e.g. parallel, crossover, non-RCT)*	
19. **Unit of allocation** *(by individuals, cluster/ groups or body parts)*	
20. **Total no. randomised** *(or total pop. at start of study for NRCTs)*	
21. **Withdrawals and exclusions (if not provided below by outcome)**	
22. **Age**	
23. **Sex**	
24. **Race/Ethnicity**	
25. **Severity of illness**	
26. **Other relevant sociodemographics**	

Intervention

27. **Group name**	
28. **No. randomised to group** *(specify whether no. people or clusters)*	

29. **Description** *(include sufficient detail for replication, e.g. content, dose, components; if it is a natural experiment, describe the pre-intervention)*	
30. **Duration of treatment period**	
31. **Timing** *(e.g. frequency, duration of each episode)*	
32. **Providers (e.g. no., profession, training, ethnicity etc. if relevant)**	
33. **Setting**	

Outcome

34. **Outcome name**	
35. **Time points measured** *(specify whether from start or end of intervention)*	
36. **Person measuring/ reporting**	
37. **Unit of measurement (if relevant)**	
38. **Scales: upper and lower limits (indicate whether high or low score is good)**	
39. **Imputation of missing data (e.g. assumptions made for ITT analysis)**	
40. **Assumed risk estimate (e.g. baseline or population risk noted in Background)**	

Results (dichotomous: odds ratio, risk ratio and confidence intervals, p-value)

41. **Comparison**	

42. **Outcome**				
43. **Subgroup**				
44. **Time point** *(specify whether from start or end of intervention)*				
45. **Results** *Note whether:* **...** *post-intervention OR* **...** *change from baseline And whether* **...** *Adjusted OR* **...***Unadjusted*	**Intervention**		**Comparison**	
	No. events	No. participants	No. events	No. participants
46. **Baseline data**	**Intervention**		**Comparison**	
	No. events	No. participants	No. events	No. participants
47. **No. missing participants and reasons**				
48. **No. participants moved from other group and reasons**				
49. **Any other results reported**				
50. **Unit of analysis** *(e.g. by individuals, health professional, practice, hospital, community)*				
51. **Statistical methods used and appropriateness of these methods** *(e.g. adjustment for correlation)*				

Results (continuous: mean difference, confidence intervals)

52. **Comparison**	
53. **Outcome**	
54. **Subgroup**	
55. **Time point** *(specify whether from start or end of intervention)*	

56. **Post-intervention or change from baseline?**						
57. **Results** *Note whether:* **...** *post-intervention OR* **...** *change from baseline* *And whether* **...** *Adjusted OR* **...***Unadjusted*	**Intervention**			**Comparison**		
	Mean	SD (or other variance)	No. participants	Mean	SD (or other variance)	No. participants

58. **Results** *Note whether:* **...** *post-intervention OR* **...** *change from baseline* *And whether* **...** *Adjusted OR* **...***Unadjusted*	**Intervention**			**Comparison**		
	Mean	SD (or other variance)	No. participants	Mean	SD (or other variance)	No. participants
59. **Baseline data**	**Intervention**			**Comparison**		
	Mean	SD (or other variance)	No. participants	Mean	SD (or other variance)	No. participants

60. **No. missing participants and reasons**		
61. **No. participants moved from other group and reasons**		
62. **Unit of analysis** *(e.g. by individuals, health professional, practice, hospital, community)*		

63. **Statistical methods used and appropriateness of these methods** *(e.g. adjustment for correlation)*	

Applicability

64. **Have important populations been excluded from the study?** *(consider disadvantaged populations, and possible differences in the intervention effect)*	... *Yes/No/Unclear*	
65. **Is the intervention likely to be aimed at disadvantaged groups?** *(e.g. lower socioeconomic groups)*	... *Yes/No/Unclear*	
66. **Does the study directly address the review question?** *(any issues of partial or indirect applicability)*	... *Yes/No/Unclear*	
67. **Key conclusions of study authors**		
68. **References to other relevant studies**		

Appendix B: Critical appraisal risks programme tool

Paper for appraisal and reference:.....

Section A: Are the results of the trial valid?

1. Did the study address a clearly focused issue?

Yes ☐
Can't Tell ☐
No ☐

HINT: An issue can be 'focused' In terms of
- the population studied
- Whether the study tried to detect a beneficial or harmful effect
- the risk factors studied

Comments:

2. Did the authors use an appropriate method to answer their question?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider
- Is a case control study an appropriate way of answering the question under the circumstances
- Did it address the study question

Comments:

Is it worth continuing?

3. Were the cases recruited in an acceptable way?

Yes	
Can't Tell	
No	

Comments:

HINT: We are looking for selection bias which might compromise validity of the findings

- are the cases defined precisely
- were the cases representative of a defined population (geographically and/or temporally)
- was there an established reliable system for selecting all the cases
- are they incident or prevalent
- is there something special about the cases
- is the time frame of the study relevant to disease/exposure
- was there a sufficient number of cases selected
- was there a power calculation

4. Were the controls selected in an acceptable way?

Yes	
Can't Tell	
No	

Comments:

HINT: We are looking for selection bias which might compromise the generalisability of the findings

- were the controls representative of the defined population (geographically and/or temporally)
- was there something special about the controls
- was the non-response high, could non-respondents be different in any way
- are they matched, population based or randomly selected
- was there a sufficient number of controls selected

5. Was the exposure accurately measured to minimise bias?

Yes	
Can't Tell	
No	

HINT: We are looking for measurement, recall or classification bias

- was the exposure clearly defined and accurately measured
- did the authors use subjective or objective measurements
- do the measures truly reflect what they are supposed to measure (have they been validated)
- were the measurement methods similar in the cases and controls
- did the study incorporate blinding where feasible
- is the temporal relation correct (does the exposure of interest precede the outcome)

Comments:

6. (a) Aside from the experimental intervention, were the groups treated equally?

HINT: List the ones you think might be important, that the author may have missed

- genetic
- environmental
- socio-economic

List:

6. (b) Have the authors taken account of the potential confounding factors in the design and/or in their analysis?

Yes	
Can't Tell	
No	

HINT: Look for

- restriction in design, and techniques e.g. modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors

Comments:

Section B: What are the results?

7. How large was the treatment effect?

HINT: Consider
- what are the bottom line results
- is the analysis appropriate to the design
- how strong is the association between exposure and outcome (look at the odds ratio)
- are the results adjusted for confounding, and might confounding still explain the association
- has adjustment made a big difference to the OR

Comments:

8. How precise was the estimate of the treatment effect?

HINT: Consider
- size of the p-value
- size of the confidence intervals
- have the authors considered all the important variables
- how was the effect of subjects refusing to participate evaluated

Comments:

9. Do you believe the results?

Yes ☐
No ☐

HINT: Consider
- big effect is hard to ignore!
- Can it be due to chance, bias, or confounding
- are the design and methods of this study sufficiently flawed to make the results unreliable
- consider Bradford Hills criteria (e.g. time sequence, does-response gradient, strength, biological plausibility)

Comments:

Section C: Will the results help locally?

10. Can the results be applied to the local population?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider whether
- the subjects covered in the study could be sufficiently different from your population to cause concern
- your local setting is likely to differ much from that of the study
- can you quantify the local benefits and harms

Comments:

11. Do the results of this study fit with other available evidence?

Yes ☐
Can't Tell ☐
No ☐

HINT: Consider
- all the available evidence from RCT's Systematic Reviews, Cohort Studies, and Case Control Studies as well, for consistency

Comments:

Remember One observational study rarely provides sufficiently robust evidence to recommend changes to clinical practice or within health policy decision making. However, for certain questions observational studies provide the only evidence. Recommendations from observational studies are always stronger when supported by other evidence.